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## THE COXSACKIE GROUP OF VIRUSES

### EPIDEMIOLOGICAL STUDIES

BY

**PIRKKO POHJANPELTO**

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MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

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# PREFACE

Docent N. Oker-Blom, M.D., Chief of the Bacteriological Laboratory of Helsinki started Coxsackie virus investigations in Finland in 1951. At his suggestion I began in autumn 1951 to study the epidemiology of the Coxsackie group of viruses. Docent Oker-Blom has supervised my work at its various stages and given me unstinted support and encouragement throughout the course of the work.

I have carried out my work in the Department of Virology, which during these early years of its activities has been connected to the Department of Serology and Bacteriology of the University of Helsinki. Professor K. O. Renkonen M.D., chief of the Department of Serology and Bacteriology has made my work possible by placing at my disposal test animals and technical assistance. It has been a pleasure to work in the stimulating scientific atmosphere created by him.

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Dr. E. Saxén M.D. has kindly carried out the histological examinations.

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The sera from healthy subjects I have collected in co-operation with Dr. S. Pere M.D. Docent E. Helske, M.D., Dr. J. Lehtinen,

M.D., Dr. E. Estola, M.D., and the Blood Bank of the Finnish Red Cross, chief Dr. H. Nevanlinna M.D.

Mrs. Airi Åberg has given me skilful help with the virus isolations and the neutralisation tests. Mrs. Aili Loukimo and Mrs. Pirkko Koivunen have given their competent assistance in the complement fixation tests.

Mrs. Hilkka Kontiopää, M.A. (Helsinki) and Mr. L. A. Keyworth, M.A. (Cantab) have translated my paper into English.

Dr. E. Kaila, Ph. D., has helped me with the statistical problems.

I wish to express my thanks and gratitude to all those who have in various ways assisted me.

*Pirkko Pohjanpelto.*

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# INTRODUCTION

Coxsackie viruses were discovered by sheer coincidence in connection with a poliomyelitis investigation. In the autumn of 1947, Dalldorf and Sickles were experimenting with the cultivation of the poliomyelitis virus in new-born mice by injecting the faeces of poliomyelitis patients into them. Two out of ten faecal samples induced paralysis in the mice. It was soon discovered, however, that the cause of the paralysis was not the poliomyelitis but an entirely new virus. It was named Coxsackie after the small village in New York State where it was first isolated.

Ever since Dalldorf published his observations the Coxsackie virus has been the subject of intense investigation. It was early established that the question was not one of a single virus but of a whole group of viruses with a number of common characteristics but differing serologically. These viruses have been reported on in numerous papers from various parts of the world and evidently quite a common and wide-spread virus group is involved.

In the early stage of the investigation, Dalldorf stated »We are in the anomalous situation of having discovered the cause of the disease before discovering the disease». Subsequently Coxsackie viruses have been found in connection with several different diseases. The part they play in inducing diseases is, however, not quite clear, nor is it known whether the various types differ in this respect. As none of the usual run of test animals react to Coxsackie infection exactly like man, and since large-scale experiments on humans are out of the question the solution to these problems can only be sought through epidemiological investigations. The present investigation is an attempt to contribute towards the solution of the problem.

# SURVEY OF THE LITERATURE

## CHARACTERISTICS OF THE VIRUS

### DEFINITION OF COXSACKIE VIRUSES

The first Coxsackie virus isolated by Dalldorf induced paralysis in newborn mice and hamsters but had no effect on adult mice and hamsters. It produced no symptoms in the monkeys and guinea pigs also injected with it, nor was it possible to reproduce it in the developing chick embryo. The virus caused degeneration in the striated muscles of newborn mice (29, 30). The changes are not, however, peculiar to Coxsackie viruses; similar changes are produced in hamsters by the MM virus and in mice by the Theiler virus. They can also be seen in experimentally induced Vitamin C or E deficiency (24).

The distinguishing characteristic of the Coxsackie virus seemed to be its peculiar host range, and particularly the fact that the age of the animal was of decisive importance.

Later it was discovered that some Coxsackie types at least could live in hosts other than suckling mice and hamsters and that the effect of the different types on mice of varying ages differed to some extent. It was also found that the pathologic picture induced by the different types in suckling mice was somewhat different. But a characteristic common to all Coxsackie types is that *suckling mice are more susceptible to infection than the adult animals and that suckling mice, as symptoms of infection, show flaccid or spastic paralyses and degenerative muscular changes.*

### VIRUS TYPES

Sixteen serologically different Coxsackie types have been distinguished to date by neutralization and complement fixation

methods. Laboratories employ different names, which makes comparisons of the types difficult.

Dalldorf distinguished 14 types. He divided them into two main groups, A and B, according to the pathologic picture they produce in suckling mice. The viruses of Group A damage the muscular tissue only, while those of Group B induce changes in the muscles, the central nervous system, adipose tissue and organs of the abdominal cavity. The Group A viruses, total ten, are numbered from one to ten. The Group B viruses are four, numbered one to four (39). There seems to be every justification for this division into Groups A and B and the names are easy to remember. It is these names that will be used in the present paper.

Melnick and co-workers have distinguished 16 types, generally named after the place where they were isolated (17).

Huebner and co-workers have distinguished 7 types, also with special names (3).

Melnick and co-workers have compared the above types and found that the types isolated in different laboratories correspond to one another (17) (Table 1). The different types generally differ distinctly, i.e. each reacts to its own immune serum only. A slight

TABLE 1

THE CORRESPONDENCE OF COXSACKIE TYPES ACCORDING TO CONTRERAS AND BARNETT AND MELNICK (17)

| Dalldorf | Melnick | Huebner |
|---|---|---|
| A 1 | Easton —2 | Type 1 |
| A 2 | Type 2 (Fleetwood) | Type 2 |
| A 3 | Type 3 (Olson) | — |
| A 4 | Texas —1 | Type 4 |
| A 5 | Easton —14 | H 1 |
| A 6 | Israel —7 | H 4 |
| A 7 | Texas —15 | — |
| A 8 | Easton —10 | H 2 |
| A 9 | Boston | — |
| A 10 | Alaska —5 | H 3 |
| B 1 | Conn —5 | — |
| B 2 | Ohio —1 | — |
| B 3 | Nancy | — |
| B 4 | Texas —13 | — |
| — | Texas —12 | — |
| — | Texas —14 | — |

cross reaction has been observed between the following types: B 1—B 3, Texas 14—B 3, Texas 12—A 5, A 3—A 8. Heterologous immune serum, however, neutralizes only approx. 100 $ID_{50}$ viruses while homologous immune serum neutralizes approx. 10,000—100,000 $ID_{50}$ (4, 17).

In addition to the types mentioned, Coxsackie strains named independently and not compared with the types listed above have been isolated in several laboratories. It is possible, therefore, that there may be more than 16 Coxsackie types.

Nothing definite is known so far of how close the relationship is between the different Coxsackie types. Hence the properties established for one type cannot simply be given a general application covering the viruses of the Coxsackie group.

## PATHOLOGIC PICTURE

Only the pathologic features produced by Coxsackie viruses in suckling mice will be discussed as they alone have been studied in detail.

The pathologic features produced by all the virus types of *Group A* are highly similar. The muscle fibres lose their finer structure, hyaline degeneration, fragmentation and clumping occur. The lesions are confined to the striated muscles. The changes are seen over extensive areas and may appear in any striated muscle.

The viruses of *Group B* induce changes in the central nervous system, adipose tissue and some internal organs as well as in the muscles. The *muscular lesions* are similar in type to the Group A lesions but only occur sporadically in small foci. In the *brain* encephalomyelitis is observed. Clearly defined necrotic areas are seen both in the grey and white matter. They appear above all in the area of the brain stem, but often in the spinal cord too. Inflammatory changes around the necrotic areas are rare. The *adipose tissue* shows necroses which usually start from the periphery of the lobules and continue towards the centrum. Inflammatory signs are seen around the necrotic areas. These lesions are mostly found in the adipose tissue between the shoulder-blades. The *internal organs*, liver, pancreas, heart and kidneys are injured considerably less regularly than the above tissues. In this respect there may be differences between the types and it is possible that the strain of mice employed also plays a part (39, 41).

## PHYSICAL AND CHEMICAL PROPERTIES

Beautiful pictures of Coxsackie virus, types A 2, A 10 and B 3 have been made under the electron microscope (8, 9). These viruses are spherical particles which are notably uniform. The particles form clusters which are hexagonal or diamond arrays. Type A 10 formed pseudocrystals in 2—3 superimposed plates.

The Coxsackie viruses are among the very smallest of viruses. Types A 1, A 4, B 1 and B 2 have been studied by the ultrafiltration and sedimentation method. Their recorded size is 10—32 mm$\mu$ (42, 61, 77, 89). From electron microscope pictures the diameter of Types A 2, A 10 and B 3 have been given as 37 mm$\mu$ (8). The types do not seem to differ appreciably in size, though Type A 1 is believed to be smaller than the others (77).

The Coxsackie viruses are fairly resistant to heat and pH changes. Types A 1, A 2, A 3 and B 1 survive for 30 min. at +49°C (93), and a virus (type not mentioned) of Group A survived 7 days at room temperature and several months at +4°C (3). Types A 1, A 2, A 3 and B 1 keep for 24 hours at pH 2.3—pH 9.4, and for 7 days at pH 4.0—pH 8.0. Of these types, A 1 was more sensitive to acid and A 3 more sensitive to alkali than the others (30). No systematic investigation has been made into the effect of disinfectants on the various types but it seems that the Coxsackie viruses are insensitive to ether, lysol and ethyl alcohol but sensitive to 0.1 normal hydrochloric acid and formaldehyde (67, 100). Type B 2 differs from the others in being sensitive to ether.

## HOST RANGE

Coxsackie viruses have been found in man, but they apparently also occur in some animals as well. O'Connor and Morris isolated a Coxsackie virus of Type A 4 from the blood of a wild rabbit (16). Melnick and co-workers were able to recover Coxsackie viruses from flies. No evidence was obtained, however, of Coxsackie viruses propagating in flies (68, 76). The examination of a large number of wild individuals of different animal species revealed the presence of antibodies neutralizing A 1 and/or A 7 and/or A 3 and/or A 4 in several rabbits, one marmot and one fox (81). In addition, A4 neutralizing antibodies have been established in four monkeys imported direct from Africa. In spite of the antibodies, however,

these monkeys became virus carriers after they had been fed with virus of Type A 4 (70).

In the laboratory it has been possible to propagate Coxsackie viruses in several experimental animals and some strains also in the developing chick embryo and tissue cultures. In most cases, however, the Coxsackie virus has been apathogenic or only slightly pathogenic.

Fatal infection is induced by Coxsackie viruses in suckling mice and suckling hamsters. Similarly two Coxsackie strains isolated in France (type not determined) were found highly pathogenic to suckling gerbils, an animal of the rodent class (62).

*In adult mice* the Coxsackie viruses usually induce no disease. An exception is Type B 1. It may damage the pancreas, liver and adipose tissue of adult mice but has not been found to produce muscular changes (44, 60, 85). The sensitivity of mice to this virus shows a wave-like fluctuation. It is greatest at birth, decreasing during the first week of life. After that it increases again to a second peak at the age of 3—4 weeks, to decrease gradually afterwards (44). However, the pathogenicity of the different B 1 strains for adult mice seems to vary. An interesting observation has been made: a virus strain transferred in suckling mice from pancreas to pancreas induced lesions in the pancreas of adult mice too, whereas another strain transferred in suckling mice from brain to brain had no such effect (28). Cortisone treatment has been found to increase the sensitivity of mice to Coxsackie. For instance, young mice weighing 7 g were found to grow more sensitive to viruses A 2, A 3, A 4 and B 2 after cortisone administration. Only types B 1 and B 3, however, were fatal to 15 g adult mice (55).

Coxsackie virus injected into *monkeys* or *suckling rats* does not produce disease in the animals but makes them virus carriers. Monkeys show a rise in antibodies after infection, and small areas of the muscles of suckling rats reveal changes similar in nature to those of suckling mice (6, 70).

The first Coxsackie strain isolated by Dalldorf did not grow in the *developing chick embryo* (30). But types A 2, A 4, A 8 and A 10 of the viruses discovered later have been made to propagate in the chick embryo (9, 40, 49, 94). Yet differences on this point may appear even between virus strains identical in type. Usually, Coxsackie infection does not kill the chick embryo.

It has been possible to cultivate several Coxsackie strains in human and animal *tissues in vitro*. The De Mole strain of Group B was grown in human embryonal brain and intestinal tissue and mature kidney, but not in embryonal cutaneous and muscular tissue. Type A 4 grew in embryonal brain tissue only (111). Type B 1, but not A 1, could be made to grow in the muscular tissue and adipose tissue of newborn mouse (99).

### SEROLOGIC REACTIONS

Coxsackie antibodies can be shown both by the neutralization and by the complement fixation method (56, 73).

In the *neutralization test* the experimental animals employed are suckling mice. The neutralizing antibodies seem to be specific in mice and monkeys but not quite specific in men (4, 73). The application of the neutralization test is restricted by the heavy consumption of mice.

In the *complement fixation test* a virus suspension purified with acetone-ether (12), protamine (18), bentonite (82) or by freezing and melting (18) is employed as the antigen. Complement fixing antibodies are specific in mice (19), but Coxsackie-infected monkeys show a rise in both homologous and heterologous Coxsackie antibodies (58). The same phenomenon is found with man (4, 57). The advantage of the complement fixation test is that a large number of sera can be examined in a short time and considerably fewer mice are needed.

*Hemagglutination.* — The effect of types A 1 and A 2 on human 0 cells and on the blood corpuscles of sheep, chicken and guinea pig at different temperatures has been tested (13). The effect of the C 2 strain, isolated in England, on human 0 cells has also been studied (32). All the hemagglutination tests gave a negative result.

## EPIDEMIOLOGY

### VIRUS SOURCES

Coxsackie viruses have been found above all in man but they have also been isolated from sewage and flies (14, 68).

The richest human source of virus is the *faeces*. Viruses are

found in stools in the earliest days of the disease, and they continue to be found regularly for a run of weeks (15). They may persist still longer and have sometimes been found for a period of several months, a couple of times for 10—15 months even (5).

Coxsackie viruses can be isolated from *throat washings* in the early stage of the disease only. It has been found, however, with monkeys, that virus B 2 can be isolated over a longer period from throat washings than from the faeces (70).

Three authors succeeded in isolating the Coxsackie virus from the *blood*. Howitt examined 39 blood samples and isolated the Coxsackie virus in 26 of them. This result, however, must be accepted with reserve as paralysis was observed in the course of the work also in mice injected with normal mouse tissue (45). From twenty blood samples examined in Switzerland two Coxsackie strains were isolated. In both cases the blood had been taken from the patient on the 5th day of the disease (5). Finally, in Egypt the virus was found in 10 out of 1144 blood samples taken from patients with an acute fever disease (103). These reports suggest that viremia may be present in connection with Coxsackie infection. However, it seems to be difficult to demonstrate the virus in blood as other laboratories have been unsuccessful in their attempts to isolate it (4, 27).

*Cerebrospinal fluids* have been studied by several laboratories but once only has a Coxsackie virus been isolated (37).

Howitt has isolated from *cerebral and cerebrospinal tissue* viruses of types A 2, A 4 and A 5, a total of 7 virus strains (45). Unfortunately this report cannot be considered fully reliable either because of the laboratory infections mentioned above.

It has been possible to isolate a Coxsackie virus of undefined type from the *muscle* of two patients with epidemic myalgia. A virus of the same type was isolated from the faeces of the same patients (63).

### GEOGRAPHICAL INCIDENCE OF VIRUSES

Coxsackie viruses appear to be universal. They have been found in all the five continents and at practically all latitudes (Table 2).

Study of the spread of the different types reveals that viruses of types A 2 and A 1 are the most commonly found and A 9, B 2, B 4, Texas 12 and Texas 14 occur less frequently (Table 3). But the table

TABLE 2

GEOGRAPHICAL INCIDENCE OF COXSACKIE VIRUSES

| Country | No. of Coxsackie isolations | Types |
|---|---|---|
| *Europe* | | |
| Denmark (65) ..... | 3 | ? |
| England (10, 11, 31, 108) | 29 | B |
| Finland (84) ..... | 3 | A10 |
| France (80) ..... | 5 | A1, A2 |
| Germany (107) ..... | 20 | A2, B |
| Hungary (50) ..... | 1 | ? |
| Iceland (104) ..... | 9 | ? |
| Italy (1) ..... | 1 | ? |
| Netherlands (106) .... | 20 | A1, A2, A3 |
| Sweden (37, 52) ..... | 125 | A4, A5, A8, A10, B3 |
| Switzerland (5) ..... | 48 | ? |
| *The Americas* | | |
| Alaska (2) ..... | 2 | A10 |
| Brazil (105) ..... | 40 | A5, A10, B |
| Canada (92, 113) ..... | 21 | A, B |
| U.S.A. (7, 15, 17, 27, 59, 61) ..... | 360 | A1, A2, A3, A4, A5, A6, A7, A8; A9, A10, B1, B2, B3, B4, Texas 14, Texas 12 |
| *Australasia* | | |
| Australia (98)..... | 15 | A4, B |
| New Zealand (33)..... | 1 | ? |
| *Asia* | | |
| Japan (34) ..... | 6 | A1, A4 |
| *Africa* | | |
| Egypt (17) ..... | 10 | A2, A3, A4, A6, A7, A10, B3 |
| Israel (64) ..... | 15 | A1, A3, A4, A7, A8 |
| Transvaal (66, 87)..... | 14 | B4 |

can hardly be considered to give a correct picture of the general incidence of the different types for three-quarters of the viruses listed were isolated in the United States.

TABLE 3

THE INCIDENCE OF DIFFERENT COXSACKIE TYPES FROM 1947 TO 1951 ACCORDING TO THE LITERATURE

| Type | —47 | —48 | —49 | —50 | —51 | Total |
|---|---|---|---|---|---|---|
| A 1 | 3 | 9 | 66 | 2 | 4 | 88 |
| A 2 | 3 | 11 | 28 | 2 | 52 | 96 |
| A 3 | | 13 | 16 | | 3 | 32 |
| A 4 | | 14 | | 4 | 14 | 32 |
| A 5 | | 3 | 19 | 16 | 1 | 39 |
| A 6 | | | 11 | 12 | 6 | 29 |
| A 7 | | 2 | 1 | | 2 | 5 |
| A 8 | | 4 | 5 | 23 | 1 | 33 |
| A 9 | | | | | 1 | 1 |
| A 10 | | 4 | 4 | 29 | 15 | 52 |
| B 1 | 3 | 29 | 5 | | | 37 |
| B 2 | 3 | | 1 | | | 4 |
| B 3 | | 3 | 3 | 11 | 3 | 20 |
| B 4 | | 1 | | | 1 | 2 |
| Tex 12 | | 5 | | | | 5 |
| Tex 14 | | 2 | | | | 2 |
| Total | 12 | 100 | 159 | 99 | 103 | 473 |

## SEASONAL INCIDENCE OF VIRUSES

Most of the Coxsackie viruses have been found in the summer and autumn. Only 16 of the 276 strains isolated all over the globe, i.e. 6 per cent, were found in winter or spring. This makes a summer-autumn incidence 16 times higher than the winter-spring incidence. The ratio is probably incorrect, however, since presumably the majority of the samples investigated were collected in the summer and autumn. A better picture of the situation is obtainable from investigations in which Coxsackie viruses have been actively sought the year round. In Sweden over 1500 samples, taken in every month of the year, were examined. Of those taken in the first half of the year 2 per cent were Coxsackie-positive, in the latter half 5 per cent (37). In the United States over 2,000 faecal samples were studied. Coxsackie viruses were found in 10 per cent of the samples collected in August—September and in 2 per cent of those collected in the other months (15). However, in this investigation the samples were not taken every month of the year. Results of tests or specimens of sewage collected each month from different areas of the United

States also indicate that Coxsackie viruses are encountered more frequently and in greater quantities during the summer and autumn (68).

### INCIDENCE OF VIRUSES AT DIFFERENT AGES

Coxsackie viruses have mostly been isolated from children. Only 69, i.e. 27 per cent, of the 285 virus strains isolated in different parts of the world were found in persons over 10 years of age. Out of over 1,000 samples studied in the United States Coxsackie virus was found in 10 per cent from persons under 10 but in only 4 per cent of those over 10 (15).

### IMPORTANCE AS PATHOGENIC AGENTS

The first Coxsackie viruses were isolated from the faeces of poliomyelitis patients. In the search for further strains there was a natural tendency to concentrate on suspected cases of poliomyelitis Coxsackie viruses were in fact found in both paralytic and the so-called non-paralytic poliomyelitis. But they were also isolated from cases of meningitis and encephalitis not associated with any poliomyelitis epidemic. Reports were soon received on the occurrence of Coxsackie viruses in connection with other diseases as well. They were found in patients affected with epidemic myalgia, herpangina and minor illnesses, but also in symptom-free persons.

All the above diseases are fairly heterogeneous in their symptoms. However, frequent symptoms are fever, headache, muscular pain and a sore throat.

It is difficult to assess the pathogenic significance of the Coxsackie group since there are so many types and since Coxsackie viruses are often found together with poliomyelitis viruses.

*Poliomyelitis.* — The relationship of Coxsackie viruses to poliomyelitis is interesting. The occurrence of Coxsackie and poliomyelitis viruses is similar in many respects. Both have been found in sewage and flies, occur most frequently in late summer, are most readily isolated from faeces and the majority of those infected are children. The two viruses are encountered together and have frequently been isolated from the same patient. They have similar

physical and chemical properties too. They are relatively small in size, and are fairly resistant to heat and to pH changes. It is inevitable to wonder whether the periodic co-occurrence of Coxsackie and poliomyelitis viruses is a coincidental result of their similarity or whether they are inter-dependent in some way.

The problem may be viewed from two angles. Firstly, the frequency with which Coxsackie viruses are found in connection with poliomyelitis; secondly, the frequency with which poliomyelitis virus can be isolated from patients affected with a Coxsackie virus. All the cases of poliomyelitis reported in the literature in which Coxsackie examinations have been made have been collected in tabulated form (Table 4). Coxsackie viruses were looked for in a total of 1,709 poliomyelitis patients. 124 strains were found, which indicates that 7 per cent had Coxsackie infection. This figure, however, includes non-paralytic poliomyelitis, of which the diagnosis is often uncer-

TABLE 4

THE OCCURRENCE OF COXSACKIE VIRUSES IN CLINICAL POLIOMYELITIS

| Author | Diagnosis | No. of poliom. cases examined | No. of Coxsackie isolations | Type |
|---|---|---|---|---|
| Curnen *et al.* (21).... | P.P. | 6 | 2 | A1 |
| » .... | N.P. | 13 | 0 | |
| Curnen (20) ..... | P.P. | 6 | 0 | |
| » ..... | N.P. | 13 | 5 | ? |
| Dalldorf (27) ..... | P. | 517 | 30 | A1, A2, A3, B |
| Gard *et al.* (37) .... | P.P. | 396 | 4 | ? |
| Levine *et al.* (64) .. | P. | 111 | 10 | ? |
| Melnick *et al.* (71) .. | P.P. | 28 | 21 | A1, A5, A8 |
| » .. | N.P. | 8 | 5 | A1, B3 |
| Melnick *et al.* (75) .. | P. | 4 | 1 | ? |
| Oker-Blom *et al.* (83) | P.P. | 5 | 1 | A10 |
| Parrott *et al.* (86) .. | N.P. | 429 | 15 | A, B |
| Silverthorne *et al.* (96) | P. | 6 | 1 | ? |
| Stanley *et al.* (98) .. | P. | 54 | 5 | ? |
| Travassos *et al.* (105) | P. | 59 | 19 | ? |
| Verlinde *et al.* (106) | P.P. | 54 | 5 | A1, A2 |
| | | 1.709 | 124 | |

P.P. = paralytic poliomyelitis
N.P. = nonparalytic poliomyelitis
P. = poliomyelitis

tain. But even confining the calculation to paralytic cases, the result does not change. The total becomes 495 cases; in 33, again 7 per cent, Coxsackie virus was found. The percentage 7, however, is a mean obtained from several investigations of even widely diverging results. For instance, Coxsackie virus was isolated during an epidemic from 75 per cent in one investigation (71) while in another laboratory only 1 per cent of 396 poliomyelitis patients could be proved to harbour Coxsackie virus (37). The incidence of

TABLE 5

THE OCCURRENCE OF POLIOMYELITIS VIRUS IN PATIENTS FROM WHOM COXSACKIE VIRUS HAS BEEN ISOLATED

| Author | Diagnosis | No. examined | No. poliom. isolated | Cox-sackie Types |
|---|---|---|---|---|
| Banker *et al.* (2) .... | Minor illness | 2 | 0 | A10 |
| Curnen *et al.* (21) .. | P.P. | 2 | 2 | A1 |
| Galpine *et al.* (36) .. | Meningoencephalitis | 1 | 0 | B |
| Huebner *et al.* (46) .. | Minor illness | 8 | 0 | A2 |
| Measroch *et al.* (66) .. | Diarrhoea | 1 | 0 | ? |
| » .. | Healthy subjects | 4 | 0 | ? |
| Melnick *et al.* (71) .. | P. | 36 | 20 | A1, A5, A8, B. |
| Melnick *et al.* (75) .. | Summer grippe | 2 | 2 | ? |
| » .. | P. | 1 | 0 | ? |
| Melnick *et al.* (78) .. | Minor illness | 7 | 0 | A2 |
| » .. | N.P. | 1 | 1 | ? |
| Menut (80) ..... | P.P. | 1 | 1 | ? |
| » ..... | Paralysis ascendens | 2 | 1 | ? |
| » ..... | Encephalomyelitis | 1 | 1 | ? |
| » ..... | Syndroma Guillan-Barré | 1 | 0 | ? |
| Rhodes *et al.* (92) .. | Minor illness | 4 | 4 | ? |
| » .. | Healthy subject ..... | 1 | 1 | ? |
| » .. | Meningitis ..... | 1 | 1 | ? |
| » .. | Syndroma Guillan-Barré | 1 | 1 | ? |
| Silverthorne *et al.* (96) | P. | 1 | 1 | ? |
| » .. | Minor illness | 4 | 1 | ? |
| » .. | Healthy subject | 1 | 1 | ? |
| » .. | Miscellaneous | 2 | 1 | ? |
| | | 85 | 39 | |

P.P. = Paralytic poliomyelitis
N.P. = Nonparalytic poliomyelitis
P. = Poliomyelitis

Coxsackie virus in connection with poliomyelitis epidemics obviously varies, and it may even be totally absent. But further parallel studies of poliomyelitis cases and normal material are required before any definite conclusion can be drawn as to whether the Coxsackie viruses are encountered more often in poliomyelitis patients than would be expected by sheer coincidence.

Table 5 shows that the presence of poliomyelitis virus was investigated in 85 patients from which Coxsackie had been isolated. The poliomyelitis virus isolation was successful in 39 of the cases. The number of the cases examined is limited, and the majority of them were patients with symptoms suggesting the presence of poliomyelitis virus. But it should be noted that both viruses may have been isolated also from persons whose diagnosis merely indicated a minor illness. From the foregoing it cannot yet with certainty be said whether Coxsackie viruses usually occur alone or together with poliomyelitis viruses. More information is needed about the occurrence of poliomyelitis virus, above all in the Coxsackie-positive cases where the symptoms give no cause to suspect poliomyelitis.

The types of Coxsackie virus found in connection with poliomyelitis belong in the main to Group A. Two authors report finding viruses of Group B also. Neither of them, however, mentions that paralytic polio was involved. This is interesting because in experiments with mice Type B 3 was found to have a somewhat antagonistic effect on the Lansing strain of poliomyelitis virus (25). Dalldorf also draws attention to the fact that when epidemic myalgia, considered with a fair degree of probability to be caused by viruses of Group B, is current unusually little polio is encountered (26).

*Meningitis and Encephalitis.* — Coxsackie viruses have also been found in diseases with neurologic manifestations that could not be taken for poliomyelitis. The biggest group of these diseases consists of meningitis and encephalitis.

Several laboratories have isolated Coxsackie viruses from cases of serous meningitis and encephalitis (35, 36, 37, 65, 98, 106, 112). The most extensive material investigated was published in Sweden; Coxsackie virus was isolated from 15 out of 411 cases of serous meningitis, i.e. 4 per cent. In a control material of 97

cases no Coxsackie viruses at all were found. The season in which the control samples were collected and the ages of the subjects are not, however, mentioned (35).

The majority of the Coxsackie viruses present in connection with meningitis and encephalitis have not yet been typed, but the following types at least have been found: A 1, A 2, A 3 and B 3 (35, 106, 112).

Is it correct to assume that Coxsackie viruses induce meningitis and encephalitis in man? It was found in Sweden that Coxsackie viruses were commoner in meningitis patients than in the control material. In the United States one of the laboratory workers contracted an A 4 infection. It was followed by a sore throat and meningeal symptoms, headache and slight stiffness of the neck (95). In Sweden, too, a patient who contracted a B 3 infection in the laboratory complained of a stiff neck (51). Moreover, in the course of the epidemic myalgia in Sweden in 1950, meningitis was diagnosed in addition to other symptoms in several patients, and a virus of Type B 3 was isolated in connection with the epidemic. These findings suggest that Type B 3 and presumably also Type A 4 may induce meningitis in man. One question suggests itself in this connection: are perhaps the viruses of Group A, purely myotropic in mice, neurotropic in man?

*Epidemic Myalgia.* — Epidemic myalgia was first described by Daae and Homann in Norway, who called it contagious muscular rheumatism (23, 43). Subsequently data on the occurrence of a similar disease have been received from several countries, although the names employed have varied. The commonest names are epidemic myalgia, Bornholm disease and pleurodynia. The most detailed report on the clinical picture of this disease was given by E. Sylvest who in 1934 summarized the articles published up to then and described 92 cases of the disease in Denmark (102).

As the name of the disease suggests, the characteristic symptom is muscular pain. Its typical location is the lower part of the thorax and epigastrium, but it may occur elsewhere too. Fever and headache, mostly felt in the forehead, are also present. With children, however, the symptoms of epidemic myalgia are often only abdominal pain and vomiting.

Curnen and co-workers in 1949 isolated for the first time Cox-

sackie virus from a patient with epidemic myalgia (20, 22). Subsequently Coxsackie viruses have been found by several authors in connection with this disease (10, 11, 31, 35, 38, 61, 87, 95, 101, 104, 108, 110 and 106), though no very high isolation percentages have been reached. Usually it has been impossible to isolate the virus from half the examined cases even. The viruses found in connection with epidemic myalgia have not for the most part been precisely typed yet. Nine publications, however, give the virus group. In all but one of these it was Group B. Three laboratories found B 3, (31, 35, 61), one laboratory B 1 (20) and one Type A 2 (106). The last-mentioned type, however, was found in connection with a mixed epidemic of poliomyelitis and epidemic myalgia. It is possible that here the pathogenic agent was the poliomyelitis virus alone, and that in some of the patients it had produced symptoms suggestive of epidemic myalgia. The Coxsackie virus in these cases would be a coincidental additional factor only. That Coxsackie viruses were isolated in this investigation in roughly identical proportions from paralytic poliomyelitis and so-called epidemic myalgia speaks in favour of this assumption.

Evidence that epidemic myalgia is induced by Coxsackie viruses is the infection of several laboratory workers. In the United States three persons working in the laboratory contracted a B 1 infection, and two of them showed symptoms remarkably similar to epidemic myalgia. Both had thoracic pain, headache in the forehead, and fever. One laboratory assistant contracted a B 2 infection. It also resulted in thoracic pain, headache and fever, plus aches in one shoulder (95). In England a laboratory infection caused by Coxsackie virus of undefined type was observed. The symptoms were pain in the side and fever (32). In Sweden four persons working in a laboratory contracted a B 3 infection. Three of them developed a disease somewhat similar to epidemic myalgia. All felt muscular pains, two in the lower thoracic region, two had headaches and two fever (51). Further support for the assumption that the Coxsackie group induces epidemic myalgia is the fact that Coxsackie virus was isolated from samples of the muscle of two patients affected with this disease, and changes similar to those revealed by Coxsackie virus infected mice were observed in the muscle (63). It may, then, be considered fairly probable that Coxsackie viruses cause epidemic myalgia. Types B 1 and B 3 have been isolated from naturally-

incurred infections of this disease. Type A 2 has also been found in patients affected with a disease similar to epidemic myalgia but it may be a sheer coincidence. In infections caught in laboratories, types B 1, B 2 and B 3 have been found to induce symptoms of this disease. It seems, therefore, that expressly the viruses of Group B cause epidemic myalgia; it is uncertain whether the viruses of Group A have the same property.

*Herpangina.* — Herpangina was first described by Zahorsky in 1924 (114), since when there have been several reports of its occurrence. All these descriptions referred to herpangina epidemics.

The disease is encountered most generally in the late summer and primarily among children. It is characterized by vesicles in the pharynx. The course of the disease is usually very benign. It starts suddenly with fever, possibly connected with nausea, vomiting, headache and muscular pain. In a day or two greyish vesicles 1—3 mm in diameter surrounded by a red areola appear at the back of the pharynx. Their number varies from 1—20, and they are seen on the soft palate, the uvula, on the anterior and posterior pillars of the fauces, and possibly also on the posterior wall of the pharynx and on the tonsils. In addition, the pillars of the fauces and the tonsils are intensely red. On rupturing the vesicles leave shallow ulcerations; these soon heal, leaving no traces. The duration of the disease is only 2—4 days in all.

The Coxsackie virus was first discovered in connection with herpangina by Huebner and co-workers (46, 47). During their investigations of the occurrence of Coxsackie virus in Maryland in United States a herpangina epidemic broke out in the community. Coxsackie virus was isolated from 37 of the 45 herpangina patients examined. A month before the epidemic no Coxsackie viruses had been found in the locality or in the persons who subsequently had herpangina. During the epidemic the virus could be isolated from herpangina patients in 91 per cent, herpangina contacts in 39 per cent and healthy subjects in 4 per cent. It was thus the herpangina patients that showed overwhelmingly the highest virus percentage. Four patients were also subjected to bacteriological study without producing any explanation of the cause of the disease. Nor, with the same patients was a herpes virus isolation obtained. Subsequently, Coxsackie viruses were isolated

in connection with herpangina epidemics also elsewhere (59, 113). In all the investigations the percentual number of isolates is quite high.

The viruses found in connection with herpangina all belong to the group A. Types A 2, A 4, A 5, A 6, A 8 and A 10 are encountered in this disease.

Can herpangina be considered a specific disease of the Coxsackie virus? The high virus isolation percentage and the fact that no other cause of the disease could be shown suggest the answer should be in the affirmative. In addition, in a laboratory infection in Sweden the patient had a sore throat and a small greyish vesicle in the posterior pillar of the fauces, similar to that seen with herpangina patients; a Type A 4 virus was recovered from the patient's pharynx (51). On the other hand, another investigation in Sweden suggests that herpangina perhaps cannot be considered as caused by a particular pathogenic agent. This investigation covered 11 patients with symptoms suggestive of herpangina not connected with any epidemic. Coxsackie virus could be isolated from only four of them. The cases in which the virus could not be demonstrated included one case of measles, one of parotitis, and one with a haemolytic streptococcus infection. It may be assumed that in these cases the symptoms of herpangina were caused by the same agent as the principal disease (52).

*Minor Illnesses.* — There are numerous instances of the Coxsackie virus having been found in persons suffering from a minor illness (2, 27, 53, 75, 78, 90, 92, 109, 113). The patient generally has, in addition to fever, had headache, indefinite muscular pain, abdominal pain, vomiting, a sore throat or diarrhoea. These are common symptoms in epidemic myalgia and herpangina. It is possible that a number of these patients had in fact contracted herpangina, but no one thought of keeping an eye open for possible vesicles in the pharynx. It is also possible that mild forms of epidemic myalgia are included among minor illnesses.

The most extensive material studied derives from the State of Wisconsin in the United States. An epidemic broke out among boys and girls of 9—14 years of age in a summer camp; the general symptoms were fever and a sore throat but some complained of headache and a stiff neck also, i.e. obvious meningeal symptoms.

Coxsackie virus, type A 3, was isolated from 14 of the 38 who contracted the disease. Sixty-three healthy contacts were also examined and a Coxsackie virus of the same type collected from 5 (90). It is not inconceivable that herpangina was involved here. However, it must be noted that virus type A 3 has not previously been found in connection with herpangina.

The following Coxsackie types at least have been found in patients suffering from a minor illness: A 1, A 2, A 3, A 4, B 1, B 2 and B 3.

Several laboratory infections are indicative of the possibility that certain cases of minor illness unattended by pharyngeal vesicles may have a Coxsackie origin. In the United States a laboratory worker contracted a B 1 infection. It resulted in fever, headache, nausea and hyperaemic conjunctivae. Another person in the same laboratory contracted an A 4 infection and suffered from a sore throat and indefinite muscular pain (95). A virus of Type B 3 was isolated from a laboratory worker in Sweden who had fever, generalized aches and conjunctivitis. Another person from whom a virus of Type A 4 was isolated also had fever, muscular aches and, in addition, a sore throat (51).

*Diarrhoea.* — Diarrhoea has sometimes been one of the symptoms in epidemics in connection with which Coxsackie virus has been isolated (10, 11, 87). Moreover, one case has been recorded, in which a Coxsackie infection contracted in the laboratory was followed by diarrhoea (95). In spite of this there has only been one investigation into the occurrence of Coxsackie viruses in diarrhoea. In Brazil 25 children with serious gastrointestinal disturbances were investigated. Eleven of them showed Coxsackie virus in the faeces or in throat washings (105).

The data mentioned above show the possibility that at least some Coxsackie viruses induce diarrhoea in man.

## ANTIBODIES IN THE SERUM

*The Patients.* — After Coxsackie infection the patient develops both neutralising and complement-fixing antibodies, specific to each Coxsackie type (4, 57). The antibodies develop irrespective of the clinical diagnosis, even in healthy virus carriers (4). In combined

Coxsackie-poliomyelitis infections virus antibodies against each virus have been found to develop (69).

*Neutralising antibodies* appear in the blood probably within a few days of the onset of the disease and persist for over a year. In two persons with a B 2 infection antibodies were found on the 5th day after the onset of the disease; the titre reached its maximum in a month and stayed at that level for at least two months. As long as two years later antibodies were still present though the titre was low (73). Similar findings were made on examining the B 1 antibodies of 5 patients. A high antibody titre was recorded after a fortnight and antibodies could still be detected 20 months later (54). An indication of the fact that the antibodies of Group A viruses too persist for over a year is that type A 1, A 2, A 4, A 5 and A 8 antibodies of healthy subjects with no Coxsackie infection during the observation period kept an approx. constant titre throughout a year (4).

As a rule the neutralizing antibodies are specific, but an increase has sometimes been observed in heterologous Coxsackie antibodies too. For instance, after an infection caused by Type A 10 the A 2, A 4 and A 8 antibodies increased (59), after a B 1 infection there was a rise in the titre of B 3 antibodies and after a B 2 infection of B 1 antibodies (57).

*Complement-fixing antibodies* apparently develop somewhat later than the neutralizing antibodies. One patient showed no complement fixing B 1 antibodies on the 5th day of the disease although neutralizing antibodies were present. Complement-fixing B 1 and B 2 antibodies however, have been established 8 days after the onset of the disease (57).

A Coxsackie infection is followed by an increase both in the homologous and heterologous complement-fixing antibodies. There is no regularity, however, about the types of heterologous antibodies that show this rise in titre. Infections caused by viruses of Group A have been followed by the development of antibodies against Group B viruses too (57).

Clearly, in individual cases the type of Coxsackie virus that has induced the infection cannot be defined from the occurrence of complement-fixing antibodies. What may be considered probable, however, is that complement-fixing antibodies develop as a result of Coxsackie infection only. Evidence of this is that patients con-

tracting miscellaneous infections have shown no rise in the titre of complement-fixing Coxsackie antibodies (4). This is also supported by the finding that in healthy subjects from whom no Coxsackie virus could be isolated in the course of a year complement-fixing Coxsackie antibodies only decreased and did not increase during the period (4). It must also be pointed out that in the monkey, whose development of antibodies is somewhat similar to that in man, the polio virus and West Nile virus induced no development of complement-fixing Coxsackie antibodies (58).

What accounts for this rise in heterologous antibodies? Have some of the Coxsackie types a common antigen factor which appears irregularly, and which is shown only by the complement-fixation method? Or is it possible to explain the phenomenon as an anamnestic reaction as a result of previous experience of Coxsackie virus? Favouring the latter assumption is the observation that the monkeys after Coxsackie infection usually develop complement-fixing antibodies against those Coxsackie types with which they have previously been infected. On rare occasions, however, complement-fixing antibodies of short duration also develop against a Coxsackie type with which the monkeys are not known to have had any contact (58).

*Normal Population.* — Both neutralizing and complement-fixing Coxsackie antibodies are found in healthy subjects, though without any clear correlation. Neutralising antibodies may be present although there are no complement-fixing antibodies, and vice versa (4, 57). This is understandable, bearing in mind that the latter persist longer than the former and that Coxsackie infection is often followed by a rise in the titre of heterologous complement-fixing antibodies but only seldom of heterologous neutralizing antibodies.

Coxsackie antibodies seem to be relatively common in healthy subjects in many parts of the world. In the state of Washington, United States, neutralizing A 2 antibodies were found in 77 per cent of a material of 158, but A 1 antibodies in 9.4 per cent only (46). A 5, A 6, A 8 and A 10 antibodies were all found in one or another of a material of 10 persons (4). In the state of Connecticut 80 per cent of a material of 7—9 year olds showed neutralizing A 4 antibodies and 65 per cent complement-fixing A 4 antibodies (74). In Japan A 1, A 2, A 3 and B 1 antibodies were studied; all of them

were found in over 50 per cent of the persons examined (34). From Greenland neutralizing A 4 antibodies were found in approx. 90 per cent of 68 sera obtained, A 10 antibodies in approx. 70 per cent and A 1 antibodies in approx. 80 per cent. On the other hand, no B 1 or B 2 antibodies were found at all (2).

In Europe the presence of Coxsackie antibodies in healthy subjects has been investigated only in Germany and Finland. In Germany, three of the eleven persons examined showed A 1, eleven out of thirteen A 2, all nine persons A 4, and 26 out of 73 persons, i.e. 35 per cent, B 1 antibodies (107). In Finland, in a material of 72 healthy persons 25 per cent showed complement-fixing A 10 antibodies (84).

*Age Groups.* Neutralizing and complement-fixing A 4 antibodies in umbilical cord blood and sera from 248 children of different ages were examined in the United States. Neutralizing antibodies were found in 90 per cent of the umbilical cord blood samples; of the children of 6—12 months, however, only slightly over 10 per cent showed these antibodies. With advancing age the antibodies increased, and by the age of 7—9 neutralizing antibodies were present in 80 per cent of the material; for the age group 10—14 the figure was the same. The same sera were also examined by the complement-fixation method. 67 per cent of the age group 7—9 showed complement-fixing antibodies. In all the other age groups, too, fewer persons showed complement-fixing than neutralizing antibodies. A striking observation was that only approx. 27 per cent of the age group 10—14 had complement-fixing antibodies. The authors explain the matter by pointing out that persons of this age already have plenty of neutralizing antibodies to protect them against Coxsackie infection. However, the antibodies had developed so long ago that complement-fixing antibodies had had time to disappear (74).

# PRESENT INVESTIGATION

As can be seen from the survey of the literature,

— the relationship between Coxsackie and poliomyelitis has still to be defined,

— it is unknown whether herpangina is a specific Coxsackie disease,

— only one study, and that from a small material, has been made of the occurrence of Coxsackie viruses in the diarrhoea of infants.

— the concomitance of virus types and symptoms is little known,

— there is no definite information on the significance of the Coxsackie group as pathogenic agents. Most of the reports on the occurrence of Coxsackie viruses in connection with a disease have usually applied to individual cases or to small materials only,

— Germany and Finland are the only countries in Europe from which reports have been published on the study of Coxsackie antibodies,

— the development of the neutralizing and complement-fixing antibodies against viruses of Group A has not been investigated.

## OBJECT

The object of the present investigation is to study the epidemiology of the Coxsackie group of viruses. For this purpose efforts have been made to study

(1) how often the virus can be isolated (a) in diseases in which Coxsackie viruses have previously been found and (b) in a control material of healthy subjects and subjects suffering from miscellaneous diseases,

(2) how often neutralizing and complement-fixing antibodies occur (a) in patients from whom Coxsackie virus has been recovered and (b) in healthy subjects,

(3) how often the complement-fixing antibodies occur (a) in persons of different ages and (b) in different regions of Finland.

## METHODS

White laboratory mice were employed in the tests. They were obtained from four different dealers. No difference was found, however, in their sensitivity to Coxsackie. All the mice employed were sacrificed.

The specimens and virus-containing material were kept in a deep-freeze with a temperature range of —10°C— —20°C. For a few days the temperature was a couple of degrees above zero. Due to technical difficulties, especially a shortage of animals, some faecal samples had to be kept for up to 14 months before study. Sera exceeding 2 years in age were used in some of the antibody titrations.

### ISOLATION METHOD

To save mice, three stools were studied together. A spoonful of the faeces, approximately 500 mg, was taken from each of the three stools, using a different spoon for each, and placed on a Petri dish; 1.5 ml of 0.9 per cent saline solution was added. Thus the dilution of each stool was approx. 1: 6. The solution was mixed with a spoon and centrifuged in an angle centrifuge at 16,000 r.p.m. (18000 G) for 20 minutes. Some 1 ml of the supernatant was taken carefully with a Pasteur pipette, and 5 mg of streptomycine and 1000 units of penicillin were added. 0.02 ml of the resulting solution was injected intraperitoneally into mice under 24 hours of age. One litter of mice, comprising a minimum of 6 but usually 7—8 suckling animals, or two litters of a total of not less than 6, were used per solution.

The mice were observed daily for 10 days. If half the number or more of the mice had disappeared (been eaten) during this period the same solution was injected into another litter. If paralyses appeared in one or several of the animals separate solutions were prepared from each of the three stools contained in the mixture and each of the new solutions was injected into one litter of mice.

The *second passage* was made by taking a paralysed mouse, removing its intestines and head, apart from the brain which was kept, and grinding it in a mortar with quartz sand. Now 7—8 ml of 0.9 per cent saline solution was added, mixed and centrifuged at 16,000 r.p.m. (18000 G) for 20 minutes. A litter of mice aged 24—48 hours was injected with the resulting solution.

Strict sterility was observed throughout.

*The Criteria for Coxsackie Determination.* — The specimen was not considered to contain Coxsackie virus unless: (1) it was possible to isolate the virus twice in the first passage, first from the mixture of three stools and then from one of the stools contained in the mixture. Where this condition was not met a note is made to the effect. (2) The virus was shown in the second passage. (3) The virus could be typed or, where this was not possible, the pathologic changes produced in mice were typical of Coxsackie and no symptoms of disease occurred in two adult mice injected intraperitoneally with 0.2 ml of the virus solution diluted 1: 10.

*Discussion.* — The possibility of a virus deriving from the laboratory is very slender, bearing in mind that the frequency of virus isolations is only 5 per cent and that each positive stool was injected twice into mice. On the other hand, it is possible that the number of positive samples was reduced by the following factors:

(1) Prolonged storage, in the course of which the virus may have been destroyed. However, in some instances a highly active virus could still be isolated 14 months after taking the specimen.

(2) The small number of mice that could be employed per specimen. Because it was not unusual for the existence of the virus to be revealed by the paralysis of just one mouse, more mice would have added to the chances of paralysis manifestations. The mother, moreover, often eats its diseased young (cf. p. 33). Cases of paralysis may thus have passed unnoticed.

(3) If the mice showed no paralysis in the first passage the specimen was considered negative. It is possible, however, that the virus has sometimes been so weak that it could not be isolated until the second or third passage. However, in herpangina epidemics it has been possible to isolate the Coxsackie virus in the first passage from over 90 per cent of the patients. This suggests that the second passage would not have markedly increased the number of Coxsackie isolations. Moreover, many passages increase the danger of laboratory infection.

In spite of these factors it must be stated that the percentage of virus isolations is of approximately the same order as in other extensive materials including control groups.

## IMMUNE SERUM

Immune serum was prepared by injecting the adult mice with 0.2 ml of virus dilution intraperitoneally twice a week during two weeks, a total of four times. For the first injection the virus was diluted 1: 100, for the second 1: 50 and for the last two occasions 1: 10. A week after the last injection the blood of the mice was drained by severing a foreleg. The serum was stored and used as immune serum.

The complement-fixing antibody titres of the immune sera of the different Coxsackie types usually ranged from 1: 64—1: 512. The A 1 immune sera were the poorest with a titre of only 1: 8—1: 16. The highest titre was exhibited by the A 5 immune serum which showed inhibition in a dilution of 1: 1024 even.

## NEUTRALIZATION TEST

*Test Animals.* With viruses of Group A, the age of the litters employed was 1—2 days, seldom 3 days. For the Group B viruses, the litters employed were always under 24 hours old. Each litter consisted of a minimum of 5, usually 7—8 young. In the experiment with Group A viruses the mice were observed for 7 days, in studying the antibodies of Group B for 10 days.

*Virus Suspension* was prepared similarly to the antigen used for complement fixation reactions. (p. 33). Bentonite purification, however, was omitted. Virus dilution refers to the dilution of the carcasses of the infected mice, and it was always expressed in terms of the final volume of the solution. Virus solution, diluted 1: 4—1: 10, was stored in a deep-freeze. A single solution was utilized for several titrations.

*Control.* Every neutralization test included virustitration with two litters.

*The experiment* was carried out as follows: 0.1 ml of 0.9 per cent saline solution was pipetted into test tubes, and to it was added 0.05 ml of serum not inactivated. 0.15 ml of virus dilution was then poured into each serum dilution tube, after which 1000 units of penicillin and 5 mg of streptomycin were added. In the serum-virus mixture, to which the antibiotics had been added, the final dilution of serum was 1: 8. After shaking, the mixtures were allowed to stand 2 hours at room temperature. Each solution was then injected into one litter of mice. During the injections the solutions were allowed to stand in ice water.

In titrating antibodies serial tenfold dilutions were made of the virus while the serum dilution was kept constant.

*Calculation of Antibody Titre.* The antibody titre of a serum is the ratio between the titres of pure virus, i.e. virus controls, and of the virus partly neutralized by that serum, i.e. virus mixed with the serum.

For example: The titer of pure virus is $10^{-6,3}$ and that of virus mixed with serum $10^{-3,7}$. Thus the titer of the serum is $\frac{10^{-6,3}}{10^{-3,7}} = 10^{-2,7}$, and expressed as a negative logarithm of the numeral 2.7.

50 per cent morbidity was the end point of the titration and it was computed after Reed-Muench (89). Classified as diseased were both the paralysed and the dead mice. Those that had disappeared (been eaten) were also counted as diseased if at least one of the remaining animals had become paralysed or died. If the living mice were in good condition the missing animals were disregarded. If in the last-mentioned case at least half the number of the animals employed were missing the experiment was repeated.

Where 50 per cent morbidity did not fall within the virus dilutions employed it was assumed that a solution ten times weaker than that which killed some of the mice would kill no mice and. correspondingly, that a ten-fold concentration of the solution killing some of the mice would kill all the mice.

*Discussion.* — The titres obtained by the neutralization method cannot be considered absolutely reliable for the following reasons:

(1) Only 5—10 mice could be employed per test.

(2) The reading of results often had to be based on two assumptions:

(a) that, if at least one of the members of the litter had contracted the disease or had died, the animals that had disappeared (been eaten) were also diseased. There is some justification for this assumption since if a lethal dose of Coxsackie virus is injected into suckling mice the mother eats all the young before long. If, again, virus-free solution is injected the mother eats only 15 per cent of her young on an average.

TABLE 6

VIRUS TITRES

| Type | Titre | No. of titrations |
|---|---|---|
| A 1 | 5.3—6.4 | 5 |
| A 2 | 6.8—7.0 | 4 |
| A 3 | 6.5—6.8 | 2 |
| A 4 | 7.2—7.3 | 2 |
| * A 5 | 4.7—6.6 | 3 |
| A 8 | 5.5—6.0 | 4 |
| ** A 10 | 4.6—6.4 | 6 |
| B 1 | 4.3—4.4 | 5 |

Titre is given as a negative logarithm of the dilution of the virus.

* The same virus suspension was used for five months. The apparent fall in titre was observed during this period.

** The same virus suspension was used for seven months. The apparent fall in titre was observed during this period.

(b) that a tenfold concentration of the suspension killing some of the mice would kill all the mice and a tenfold dilution of the same suspension would kill no mice. The assumption is not quite correct. When the same virus solution was injected in different dilutions it was found that a tenfold concentration of the suspension killing some of the mice killed only approximately 90 per cent of the injected animals, and a tenfold dilution of the same suspension which had killed some mice left only 90 per cent of the mice healthy.

Table 6 shows the titres of each virus type. The neutralization test, however, is not as inaccurate as the table might suggest for the titres of types A 5 and A 10 at least had definitely decreased within the few months that elapsed between titrations.

### COMPLEMENT FIXATION REACTION

The complement fixation test was carried out according to the method used in this laboratory (85). To start with, 0.9 per cent saline solution was used as the diluent; from the beginning of 1954 onwards, when the majority of the titrations were effected, buffered 0.9 per cent saline solution was used. The change in diluent was not found to affect the results.

*Antigen* was largely prepared according to the method given by Melnick (18). The difference here was the use of bentonite instead of protamine to purify the antigen because the former removes the protein more efficiently. The purification method was that developed by Oker-Blom and Nikkilä in 1952 (81, 82). In parallel titrations antigens purified with protamine and bentonite gave concurring results.

The antigen was prepared as follows: $10^4$—$10^6$ $ID_{50}$ virus, depending on the titre of the strain used, was injected into mice under 24 hours of age. After some 50 per cent of the mice were paralysed or dead the balance were killed with ether. All the animals were used for the preparation of antigen. The intestines were removed, and so was the head except for the brain. The carcasses were weighed, placed in a Waring blender, twice their weight of 0.9 per cent saline solution was added, and they were allowed to mix for two minutes. Now the mixture was allowed to stand at +4°C until the next day. It was then centrifuged at 16,000 r.p.m. (18000 G) for 20 minutes. The supernatant was collected and to it was added its own volume of 1 per cent bentonite in distilled water; the mixture was shaken well and left to stand at +4°C for 30 minutes. Now the solution was recentrifuged at 2,000 r.p.m. (900 G) for 25 minutes. The supernatant, which was quite clear and almost colourless, was employed as antigen.

The antigen dilution used in all the titrations was 1: 8. A more concentrated antigen failed to give higher titres. Each new batch of antigen was checked before use by titrating it with known immune serum.

*The haemolytic system* consisted of equal parts of 2.5 per cent sheep

cells and the appropriate dilution of haemolysin, containing two full units. Sensitizing was allowed to take place at room temperature for 15 minutes.

*The complement* used was pooled guinea pig serum. It was standardized so that 0.2 ml contained 1.26 full units.

*Controls.* The following controls were included in every titration.

| | Antigen | Serum | Complement 2·1.26 f.u. | Complement 1.26 f.u. | Complement 1 f.u. |
|---|---|---|---|---|---|
| Complement control 1 | — | — | 0.2 | — | — |
| Complement control 2 | — | — | — | 0.2 | — |
| Complement control 3 | — | — | — | — | 0.2 |
| Antigen control 1.... | 0.2 | — | 0.2 | — | — |
| Antigen control 2.... | 0.2 | — | — | 0.2 | — |
| Antigen control 3.... | 0.2 | — | — | — | 0.2 |
| Serum control ..... | — | 0.2 | — | 0.2 | — |
| 0-control ..... | 0.2 | 0.2 | — | — | — |

In every control tube diluent was added so that the final volume was always the same.

*Inactivation.* All the sera were inactivated in a dilution of 1: 4. The immune sera were kept at +59°C for 10 minutes, the human sera at +56°C for 30 minutes.

*The Experiment.* Serial twofold dilutions of serum were made, beginning from dilution 1: 4. 0.1 ml of serum dilution was placed in each tube, 0.1 ml of antigen and 0.1 ml of complement dilution added. The tubes were shaken and left to stand for 15—18 hours at +4°C. (An attempt was made at fixation in 45 minutes at +37°C but the titres obtained were lower than those yielded by the other method.) After fixation, 0.2 ml of sensitized cells were added. In titrations of patient sera, in which the height of the titre was important, double quantities of all the substances were pipetted. Haemolysis was allowed to take place in a waterbath at +37°C, usually in the course of 10—20 minutes. Immediately the antigen control with the complement dilution used in the titration was clear but the other antigen control with 1: 1.26 of the complement dilution used was still slightly turbid, the tubes were removed from the waterbath. If, however, the series were left to stand in the waterbath for same length of time after the clarification of the control tubes the sera always showed very low titers or no inhibition at all. After haemolysis the tubes were placed in a refrigerator at +4°C. The results were read the following day. The supernatant on the sedimented red corpuscles was compared with the colour control achieved by adding to a tube with complete haemolysis its own volume of 0.9 per cent saline solution. The resulting colour indicated 50 per cent haemolysis, which was considered the end-point of titration.

## VIRUS TYPING

The types of the isolated virus strains were determined by the neutralization test. In some cases the result was checked by the complement fixation method.

The following 14 types of Coxsackie virus were available: A 1, A 2, A 3, A 4, A 5, A 6, A 7, A 8, A 9, A 10, B 1, B 2, B 3 and B 4. Of these, A 10 had been isolated in Finland in 1951. The other type strains were kindly placed at the laboratory's disposal by Prof. S. Gard. Satisfactory immune sera were prepared against types A 1, A 2, A 3, A 4, A 5, A 7, A 10, B 1 and B 4; the A 6 and A 8 immune sera did not work. Immune sera against A 9 and B 4 were not prepared at all as the type strains were only obtained during the final phase of the investigation.

The typing was effected by finding an immune serum to neutralize the virus. If this failed, immune serum against the unknown virus was prepared and tried out against the Coxsackie virus types for which immune sera were not available.

To save mice, three mixtures of known immune sera were made, one of them containing types A 1, A 5 and A 7, another types A 2 and A 10, and the third types B 1 and B 4. If a mixture neutralized the virus the immune sera in it were studied separately. The action of immune sera A 3 and A 4 was studied separately from the outset.

Considering the risk of laboratory infection efforts were made to determine the type at the earliest stage possible. Usually the second passage of the virus studied was employed. The third or fourth passages were only employed when the virus was weak. The unknown virus was usually not titrated but diluted if possible so that the mice fell ill on the third day after the injection. The dilution of the immune serum was always 1: 20. Otherwise the neutralization test was carried out in the same manner as for human sera.

*The Criteria of Type Determination.* — The virus was considered typed when it was neutralized by a known immune serum or when its own immune serum neutralized a known virus. Due to the limited supply of mice, however, the possibility of crossreactions between some of the immunesera and the unknown virus or between the immune serum prepared against the virus to be tested and some of the known viruses can not with certainity be excluded. The reason therefore is that the test was in several cases finished as soon as one immune serum neutralised the unknown virus, or as soon as the immune serum prepared against this virus neutralized one of the known viruses. In the cases, however, where all the immune sera were tested no crossreactions occurred.

## VIRUS ISOLATIONS

*Material.* — All the virus investigations were made with faeces. The main part of the patient samples were obtainet from The Children's Clinic (with patients from all over Finland), and The Aurora Hospital where the patients are residents of Helsinki. In addition, samples were received from Oulu, Turku, Aaland and several other localities in Finland. The normal material comprises young medical students, hospital nursing staff and persons of different ages from Aaland.

The samples were callected in 1951—1954. mostly however in 1953.

The faeces of a total of 657 persons were examined. 36 strains of Coxsackie virus were found; 29 of them were isolated twice, first from a mixture of 3 faeces, then individually from one of faeces included in the mixture. 7 strains were obtained from mixtures only. In these 7 cases the faeces either could not be examined individually due to exhaustion of the sample or failed to induce paralysis in suckling mice when injected individually. Five of these strains belonged to types not treated in the laboratory at the time of isolation; hence they apparently derived from the faeces. On the other hand, two strains, both found during the same week, belonged to a type with which extensive experiments were in progress at that time. It is questionable whether these virus strains originally existed in the samples.

Eight of the faeces studied were obtained from persons working in the laboratory or closely related to them. Coxsackie infection was found in four of them. Excluding these and the two uncertain virus isolates, the total of the samples studied is 649; 30 of them, about 5 per cent, gave Coxsackie virus isolates.

### VIRUS TYPES

Attempts were made to determine the type of all the viruses recovered. The results of typing by the neutralization method are shown in Tables 7 and 8. Strains No. 4 and 8 have not been compared with known type strains. This was considered unnecessary as their immune sera neutralized virus strain No. 1 of Type A 10. Strains No. 114, 115, 117 and 118 were compared with Type A 10 only as they were all isolated from members of one family and the virus (No. 116) recovered from one member of the family was identified with Type A 10. The typing of strains No. 10, 158, 202,

## TABLE 7

VIRUS TYPES I

| | Virus | Immune serum | | | | | | | | | Type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | Patient | A 1 | A 2 | A 3 | A 4 | A 5 | A 7 | A 10 | B 1 | B 4 | |
| 1 | J. E. | 6/6 | 5/5 | 6/6 | 5/5 | 4/4 | 4/4 | **0/6** | 5/5 | | A 10 |
| 4 | H. T. | | | | | | | | | | A 10 |
| 8 | J. K. | | | | | | | | | | A 10 |
| * 9 | P. G. | 6/6 | | 5/5 | | 6/6 | 6/6 | **0/7** | | | A 10 |
| 10 | I. L. | 5/5 | 6/6 | 8/8 | **0/5** | 5/5 | 5/5 | 6/6 | | | A 4 |
| 11 | T. R. | 7/7 | 5/5 | 5/5 | 6/6 | 7/7 | 7/7 | 5/5 | | | A R |
| 15 | M. H. | 11/11 | | 5/5 | **0/5** | 11/11 | 11/11 | 4/4 | | | A 4 |
| 26 | ? | | | | | | | | 9/9 | 9/9 | B 3 |
| 41 | T. L. | 5/5 | | | | 5/5 | 5/5 | **4/8** | | | A 10 |
| 42 | I .M. | 9/9 | | | | **0/4** | 7/7 | | | | A 5 |
| 45 | L. K. | | | | | | | | 8/8 | 5/5 | B K |
| 50 | I. L. | 10/10 | 5/5 | 7/7 | **0/5** | 10/10 | 10/10 | 5/5 | | | A 4 |
| 77 | H. H. | 6/6 | 5/5 | 7/7 | 6/6 | 6/6 | 6/6 | 5/5 | | | A R |
| 80 | ? | 8/8 | | | **0/4** | 8/8 | 8/8 | 8/8 | | | A 4 |
| 89 | P. P. | 10/10 | 7/7 | 6/6 | 6/6 | 10/10 | 10/10 | 7/7 | | | A R |
| 102 | ? | 8/8 | 7/7 | 10/10 | 10/10 | 9/9 | | 8/8 | **0/6** | 6/6 | B 1 |
| 114 | E. S. | | | | | | | **0/8** | | | A 10 |
| 115 | I. S. | | | | | | | **0/7** | | | A 10 |
| 116 | An. S. | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | **0/6** | | | A 10 |
| 117 | Ai. S. | | | | | | | **0/7** | | | A 10 |
| 118 | E. S. | | | | | | | **0/7** | | | A 10 |
| * 129 | A. L. | 8/8 | 5/5 | 6/6 | | 8/8 | 8/8 | **0/5** | | | A 10 |
| 141 | L. E. | 5/5 | 8/8 | 8/8 | 5/5 | 7/7 | 7/7 | 8/8 | 6/6 | 6/6 | A E |
| 158 | ? | 7/7 | | | **0/5** | | | 7/7 | | | A 4 |
| 167 | ? | 5/5 | | | | **0/7** | 10/10 | | | | A 5 |
| 169 | ? | | 8/8 | 7/7 | 6/6 | **0/6** | | 8/8 | | | A 5 |
| 175 | ? | | 11/11 | | **0/7** | | 11/11 | | | | A 4 |
| 202 | R. L. | 7/7 | 5/5 | 8/8 | **0/7** | 7/7 | 7/7 | 5/5 | | | A 4 |
| 228 | M. S. | | 8/8 | 8/8 | **0/9** | | | 8/8 | | | A 4 |
| 237 | T. H. | | 6/6 | | **0/7** | | | 6/6 | | | A 4 |
| 239 | M. K. | | 7/9 | | **0/6** | | | 7/9 | | | A 4 |
| 263 | V. K. | 10/10 | | | **0/4** | 10/10 | 10/10 | | | | A 4 |
| 283 | J. A. | 6/6 | 6/6 | | **0/7** | 6/6 | 6/6 | 6/6 | | | A 4 |
| 289 | M. H. | 5/5 | | | | 5/5 | 5/5 | **0/8** | | | A 10 |
| * 319 | E. T. | 7/7 | 6/6 | | | 7/7 | 7/7 | **0/6** | | | A 10 |
| * 318 | K. T. | 7/7 | 6/6 | | | 7/7 | 7/7 | **0/6** | | | A 10 |

The numerator indicates the number of diseased mice, the denominator the total mice used in the test.

* Laboratory infections.

TABLE 8

VIRUS TYPES II

| Immune serum | | Virus | | | | |
|---|---|---|---|---|---|---|
| No. | Patient | A 6 | A 8 | A 9 | B 2 | B 3 |
| 89 | P. P. | 7/7 | 10/10 | 6/6 | | |
| 141 | L. E. | 7/7 | 7/7 | 7/9 | 7/7 | |
| 26 | ? | | | | 7/8 | 0/5 |
| 1 | 3. E. | 5/5 | 8/8 | | 5/5 | |

and 114 by the neutralization test was confirmed by the complement fixation method.

Six of the 36 virus strains listed in the tables evidently derive from the laboratory. Four of them were isolated from persons working in the Virus Department or from their relatives who evidently had obtained the infection via them from the laboratory. All these strains (Nos. 9, 129, 318, 319) were of Type A 10. Another two virus strains (Nos. 167, 169), again, were probably not in the samples at all originally and really derived from the laboratory during the treatment phase. This is supported by the fact that these virus strains could only be isolated from mixtures of three faeces and not from individual samples of the faeces included in the mixtures. In addition, both were of Type A 5, the very type from which antigen was prepared at the time of their isolation.

Excluding these six strains, there remain 30 virus strains found »in nature». The commonest of them is Type A 4, of which 12 strains were found. Ten strains of Type A 10, one Type A 5, one Type B 1 and one Type B 3 were found. In addition, five isolates could not be identified with any of the types available. Three strains, denoted AR, were serologically identical. The other two strains are listed as E and BK. It is possible that they represent new Coxsackie virus types.

*Type AR* induced flaccid paralysis in suckling mice characteristic of the viruses of Group A. The titre was $10^{-7}$. It did not affect adult mice. Changes typical of Coxsackie viruses were observed in the muscles of the mice infected by this virus: disappearance of the finer structure of the muscle fibres, fragmentation and clumping. The virus differed serologically from all the Group A viruses available.

*Type E* induced flaccid paralysis in suckling mice, spasticity also sometimes. The titre in the fourth passage was $10^{-4}$. It did not affect adult mice. Changes in the muscles were similar to those induced by Type AR. This virus differed serologically from all the Coxsackie types at our disposal.

*Type BK* induced spasticity and rigid paralysis in suckling mice, i.e. symptoms typical of Group B viruses. It did not affect adult mice. The titre was $10^{-5}$. The changes it induced in the muscles of suckling mice were similar to those induced by Types AR and E. This virus differed serologically from all the Group B viruses at our disposal.

### SEASONAL INCIDENCE OF THE VIRUSES

Five of the 29 strains were found in the first half of the year, 24 in the second half. The samples examined in the former period included 3 per cent positive, those examined in the last six months 5 per cent. However, four of the viruses found in the first half of the year were isolated in June. Between January and June a single

TABLE 9

OCCURENCE OF VIRUSES, BY MONTHS

| Month | Paral. poliom. | | Meningitis encephalitis | | Herp- * angina | | Minor illness | | Diarrhoea | | Control | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. examined | No. posit. | No. examined | No. posit. | No. examined | No. posit. | No. examined | No. posit. | No. examined | No. posit. | No. examined | No. posit. | No. examined | No. posit. |
| 1 | 1 | — | 4 | — | 1 | — | — | — | 11 | — | 7 | — | 24 | — |
| 2 | 1 | — | 3 | — | 8 | — | — | — | 8 | — | 16 | — | 36 | — |
| 3 | | — | 5 | — | — | — | — | — | 6 | — | 39 | — | 50 | — |
| 4 | | — | 2 | — | 1 | 1 | — | — | 16 | — | 5 | — | 24 | 1 |
| 5 | 1 | — | 2 | — | — | — | — | — | 18 | — | 6 | — | 27 | — |
| 6 | 5 | 1 | 7 | 1 | — | — | — | — | 15 | — | 24 | 2 | 51 | 4 |
| 7 | 3 | — | 6 | — | 1 | 1 | — | — | 24 | — | 11 | — | 45 | 1 |
| 8 | 15 | — | 22 | 1 | 3 | 2 | — | — | 11 | 2 | 38 | — | 89 | 5 |
| 9 | 12 | — | 22 | 4 | 11 | 5 | 5 | — | 17 | — | 24 | — | 91 | 9 |
| 10 | 4 | — | 19 | 1 | 2 | — | 7 | 1 | 17 | 2 | 23 | — | 72 | 4 |
| 11 | 3 | — | 8 | — | 1 | — | 2 | — | 23 | 3 | 26 | — | 63 | 3 |
| 12 | 4 | — | 7 | — | — | — | — | — | 17 | 1 | 49 | 1 | 77 | 2 |
| Total | 49 | 1 | 107 | 7 | 28 | 9 | 14 | 1 | 183 | 8 | 268 | 3 | 649 | 29 |

* Three cases with both herpangina and meningitis and/or encephalitis are included in the herpangina group.

Coxsackie strain only was isolated, i.e. 1 per cent of the samples examined during this period. (Table 9.)

### INCIDENCE OF VIRUSES AT DIFFERENT AGES

Coxsackie virus was isolated from 6 per cent of the patients under 10 and 2 per cent of the patients over 10 years of age (Table 10).

TABLE 10

VIRUS ISOLATIONS IN DIFFERENT AGE GROUPS

| Diagnosis | Under 10 years | | | Above 10 years | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. examined | No. posit. | Per cent | No. examined | No. posit. | Per cent | No. examined | No. posit. | Per cent |
| Paral. poliom. ..... | 27 | — | — | 22 | 1 | 5 | 49 | 1 | 2 |
| Meningitis & encephalitis .. | 52 | 5 | 10 | 55 | 2 | 4 | 107 | 7 | 7 |
| Herpangina ..... | 16 | 7 | 45 | 12 | 2 | 17 | 28 | 9 | 32 |
| Minor illness ..... | 3 | — | — | 11 | 1 | 10 | 14 | 1 | 7 |
| Diarrhoea ..... | 183 | 8 | 4 | — | — | — | 183 | 8 | 4 |
| Control ..... | 68 | — | — | 200 | 3 | 2 | 268 | 3 | 1 |
| | 349 | 20 | 6 | 300 | 9 | 2 | 649 | 29 | 4 |

### INCIDENCE OF VIRUSES IN DIFFERENT DISEASES

The patient material comprised the following disease groups: paralytic poliomyelitis, meningitis, encephalitis, herpangina, minor illness and gastroenteritis. The control material consists of healthy subjects and miscellaneous morbid conditions.

The *control material* comprises 145 samples from healthy subjects and 123 samples from miscellaneous cases of disease. Two Coxsackie strains were isolated from the former and one from the latter group.

The healthy subjects were medical students, hospital nursing staff and persons of different ages from Aaland. One of the two carriers of Coxsackie virus found among them was a female medical student of 25 who failed to recall having suffered from any symptoms of disease for a month before and a month after the isolation of the virus. The virus recovered was of Type A 4. The other virus carrier was a woman of 31 from Aaland, a close relative of a patient affected with paralytic poliomyelitis who herself had had no illness at the time of the occurrence of the virus. The virus isolated from her was of Type A 10.

The group of miscellaneous diseases is made up as follows: pneumonia and infections of the respiratory tract 19, ulcerative stomatitis 15, tonsillitis 5, erythaema exsudativum multiforme 3, eczema subitum 2, colitis and Hirschsprung's disease 8, miscellaneous surgical and non-infectious cases of internal diseases 71. A Coxsackie virus of Type A 10 could be isolated from a man of 40 suffering from erythaema exsudativum multiforme.

*Diseases with Neurological Symptoms.* — Paralytic poliomyelitis, meningitis and encephalitis, have been placed in this group. They are discussed under one heading as a diffreential diagnosis in these cases may, be difficult. In addition, it is impossible to be certain without virus investigations which of the cases were due to poliomyelitis virus and which were not.

The cases with neurologic symptoms totalled 159; Coxsackie virus was found in 9, i.e. 6 per cent.

The patients whose clinical diagnosis was *paralytic poliomyelitis* numbered 49. One Coxsackie virus, Type A 10, was isolated from them. Thus Coxsackie infection could be established in 2 per cent of the cases.

Five of the samples studied, including the only Coxsackie-positive case of poliomyelitis, were obtained in connection with the epidemic of 1950 in Helsinki. The other samples were negative. Twenty of them were from sporadic cases in 1951, 1952 and the spring of 1953, and 24 from the epidemic in the summer and autumn of 1953.

A total of 110 patients with the clinical diagnosis *meningitis or encephalitis* were examined. Eight Coxsackie strains were found, i.e. 7 per cent of the samples studied were positive. The strains isolated were of Types A 4, A 5, A 10, AR, E and BK.

The cases of meningitis totalled 89, 6 of them Coxsackie-positive. All of them were serous meningitis with fairly clear liquor and over half the cells lymphocytes. Twenty-three of them were associated with poliomyelitis epidemics. Five of these 23 were obtained in 1950 and the balance in the autumn of 1953. Two Coxsackie strains were isolated from the earlier, none from the latter. Sixty-six cases had no connection with poliomyelitis epidemics. Five of them were tuberculous, an increase in antibodies against mumps antigen was detected in ten and against leptospira antigen in three. The remaining 53 cases were meningitis of unknown etiology; 4 Coxsackie strains were found in this group. In the last-mentioned group two

showed vesicles in the posterior part of the month and are included in the herpangina group too.

Under encephalitis were classified the cases with fever plus encephalic symptoms: disorders of consciousness, convulsions, paralysis of the cranial nerves, or ataxia. In addition, slight changes in liquor were often noted. From the total of 15 cases of encephalitis two Coxsackie strains were isolated one from the patient æith symptoms of herpangina.

*Discussion.* The majority of the cases in which the diagnosis was paralytic poliomyelitis may be assumed to have been induced by poliomyelitis virus. A considerable proportion of the cases of meningitis occurring during the poliomyelitis epidemic also probably have the same etiology.

Coxsackie virus was found in 2 per cent of the patients with paralytic poliomyelitis. The difference from the control material is only 1 per cent (dispersion 2); thus the difference must be considered not significant. Included the cases of meningitis during the poliomyelitis epidemic, the total is 72, with 4 per cent Coxsackie-positive. The difference from the control material is 3 per cent (dispersion 2) which cannot be considered significant. Hence the above material warrants no inference about the incidence of Coxsackie viruses in connection with poliomyelitis other than that it is coincidental. It is striking, however, that 3 Coxsackie strains were isolated from the 10 samples examined during the poliomyelitis epidemic in the autumn of 1951. Sixty-two cases were examined during the later epidemic but not a single Coxsackie strain could be isolated.

Coxsackie virus was isolated from 7 per cent of the patients whose diagnosis was serous meningitis or encephalitis. The difference from the control material is 6 per cent (dispersion 2.5). This difference is significant.

*Herpangina.* — If vesicles of a few millimetres in diameter were seen in the posterior part of the mouth, on the faucial pillars, uvula or tonsils the diagnosis was herpangina. Sometimes one or two vesicles were observed also elsewhere on the oral mucosa. However, all the cases were readily differentiated from stomatitis in which the

whole oral mucosa is red and inflamed. In addition to the vesicles in the mouth, the patients almost always had fever. Muscular pain and abdominal distress were also present in some cases. The disease always lasted a few days only and the vesicles healed leaving no traces.

The author examined five cases personally. Information on the other patients was received from the attending doctor or from hospital journals.

A total of 27 herpangina patients and 3 members of a herpangina patient's family who had contracted a minor illness (probably also herpangina) were examined. A total of 10 Coxsackie strains, Types A 4, A 10 and AR, were isolated. Two of the subjects with the Coxsackie infection were on the laboratory staff. If they are excluded, the total of examined cases is 28; 9 of them, 32 per cent, were Coxsackie-positive. Nine of the samples sent to the laboratory derived from military conscripts, among whom a herpangina epidemic seemed to have broken out. The remaining 19 samples were obtained from sporadic cases consulting private doctors, or from hospitals.

*Laboratory Infections.* — (1) P.G., 27, male, works permanently in the rooms where Coxsackie virus investigations are made. A tooth was extracted on Nov. 7, 1952, under infiltration anesthesia. The following night he was sweating and felt nausea. On Nov. 8 his temperature suddenly rose to 39° C. On Nov. 9 several vesicles 1—2 mm in diameter and surrounded by a 2 mm red areola were observed on the right posterior faucial pillar. In addition there was a vesicle 3 mm in diameter on the surface of the right tonsil, a couple of vesicles 1 mm in diameter on the buccal mucosa and one vesicle 1.5 mm in diameter on the interior surface of the lower lip. The pharynx was reddened but elsewhere the oral mucosa was not appreciably irritated. By Nov. 12 the fever had subsided and the vesicles been replaced by ulcerations which healed in a few days leaving no traces.

Laboratory tests: Coxsackie virus of Type A 10 was isolated from a faecal sample taken on Nov. 17. Both neutralizing and complement-fixing antibodies were found in the blood (p. 52). In the autumn of 1952, when the disease broke out, the laboratory handled Coxsackie viruses of Type A 10 only.

(2) A.L., 31, female, employed by the laboratory from Sept. 15, 1953, to carry out Coxsackie virus titrations. Sept. 26 her throat felt slightly sore. Sept. 27 the pharynx was distinctly hyperaemic. A vesicle 2 mm in diameter, surrounded by a red areola, was observed on the left anterior faucial pillar. The temperature was not taken but the patient reported feeling slightly feverish. She had a slight ache in the upper and low erextremities, accom-

panied by sudden pains of approx. 1 second's duration. The patient was not absent from work due to the disease. By Sept. 28 the subjective symptoms had disappeared. The vesicle disappeared leaving no traces.

Laboratory tests: Coxsackie virus of Type A 10 was isolated from a faecal sample taken on Sept. 28. An increase was noted in both neutralizing and complement-fixing antibodies in the blood (p. 52).

At the time of the outbreak of the disease several different types of Coxsackie virus were handled in the laboratory. On Sept. 22 the patient had carried out titrations with a Coxsackie virus strain of Type A 10.

*Epidemic.* — In 1953, at the end of January and beginning of February, there was an epidemic with symptoms of herpangina among conscripts serving on the island of Suomenlinna, off Helsinki. The symptoms were vesicles in the posterior part but nowhere else in the mouth, fever, and in some cases muscular pain, nausea and diarrhoea. The disease was of short duration and subsided leaving no traces. The strength of the unit within which the epidemic broke out was 200; about 20 contracted the disease. The exact number is uncertain as not all the patients consulted the medical officer. Immediately after this epidemic an influenza epidemic raging simultaneously in Helsinki spread to the forces on the island.

The faeces of 9 patients with herpangina was examined during the acute stage of the disease but Coxsackie virus could not be isolated from any one of them.

*The sporadic cases*, not associated with any epidemic, totalled 19. Six of them derived from the consulting rooms of private doctors, eight from hospitals, and there was a family infection of five cases.

All the samples obtained from consulting physicians derived from children under 10, all with typical symptoms of herpangina. Two Coxsackie strains, one of Type A 4 and the other of Type AR, were isolated.

Of the 8 cases treated in the hospital, two had typical herpangina, and six had some other disease in addition: two of them had tonsillitis, one slight pneumonia, one serous meningitis and two encephalitis. All of them for a few days revealed some vesicles, approximately the size of a pin-head, on the soft palate, pillars of the fauces or the posterior wall of the pharynx. The vesicles disappeared leaving no traces. The temperature began to fall in all the cases soon after the vesicles had appeared. The etiology of the disease could be ascertained in none of the cases. Two Coxsackie strains, Types A 4 and AR, were isolated from these hospital cases. The former was recovered from the patient whose additional complaint was tonsillitis, and the latter from a patient with encephalitis.

*Family infection* was observed in a doctor's family of 5; a Coxsackie virus of Type A 10 was isolated from all the members of the family. Herpangina was diagnosed in two children, the others had a minor ailment (probably herpangina).

The father was the first to sicken. He had a headache, a slight cold, and fatigue. No appreciable fever was noticed. In a few days the mother felt her throat was slightly sore, and a couple of vesicles similar to herpes

vesicles appeared on her lips. She had no fever. Thus the parents had the disease in a fairly mild form. Some 5 days after the onset of the father's illness two children, aged 4 and 5, got fever which rose to 39°C. After two days a couple of greyish vesicles surrounded by a red areola were observed at the back of the mouths of both children. One of them, in addition, had a vesicle 3 mm in diameter under the tongue. Soon after the appearance of the vesicles the fever began to drop. The total duration of the disease was approx. 4 days. The vesicles disappeared without trace. The youngest child, age 9 months, also had fever but no vesicles were observed in the mouth. The fever persisted for 3 days. The family's maidservant showed no symptoms, and no Coxsackie virus was recovered from her.

*Discussion.* The two laboratory infections resulting in symptoms of herpangina prove that Coxsackie viruses may induce the disease. This is also supported by the fact that Coxsackie viruses were found considerably more often in cases of herpangina than in other diseases or the control material.

But is herpangina always induced by the Coxsackie virus? In the United States it has been possible to isolate the Coxsackie virus from the majority of patients examined in connection with herpangina epidemics. The present investigations, however, do not support the opinion that herpangina is a specific Coxsackie disease. This virus could be isolated from only 32 per cent of the cases examined. Admittedly, detailed data were not obtainable on all the patients, and the diagnosis was often made on the basis of a single medical examination. Hence it is not certain that all the cases really were herpangina, though at least the most typical symptoms were discernible. However, the indication is that herpangina or at least a very similar condition may also be induced by factors other than Coxsackie infection. This view is supported by an investigation carried out in Sweden; only 4 Coxsackie strains could be isolated from 11 sporadic cases of herpangina and herpangina symptoms were stated in connection with infectious diseases induced by known agents other than Coxsackie virus (52).

*Diarrhoea.* — An acute condition with loose stools as the main symptom was classified as diarrhoea. Usually the patients also had fever. The severity of the disease varied from loose stools during a couple of days to severe states of intoxication. All the patients examined were under 3 years of age, the majority under 1 year.

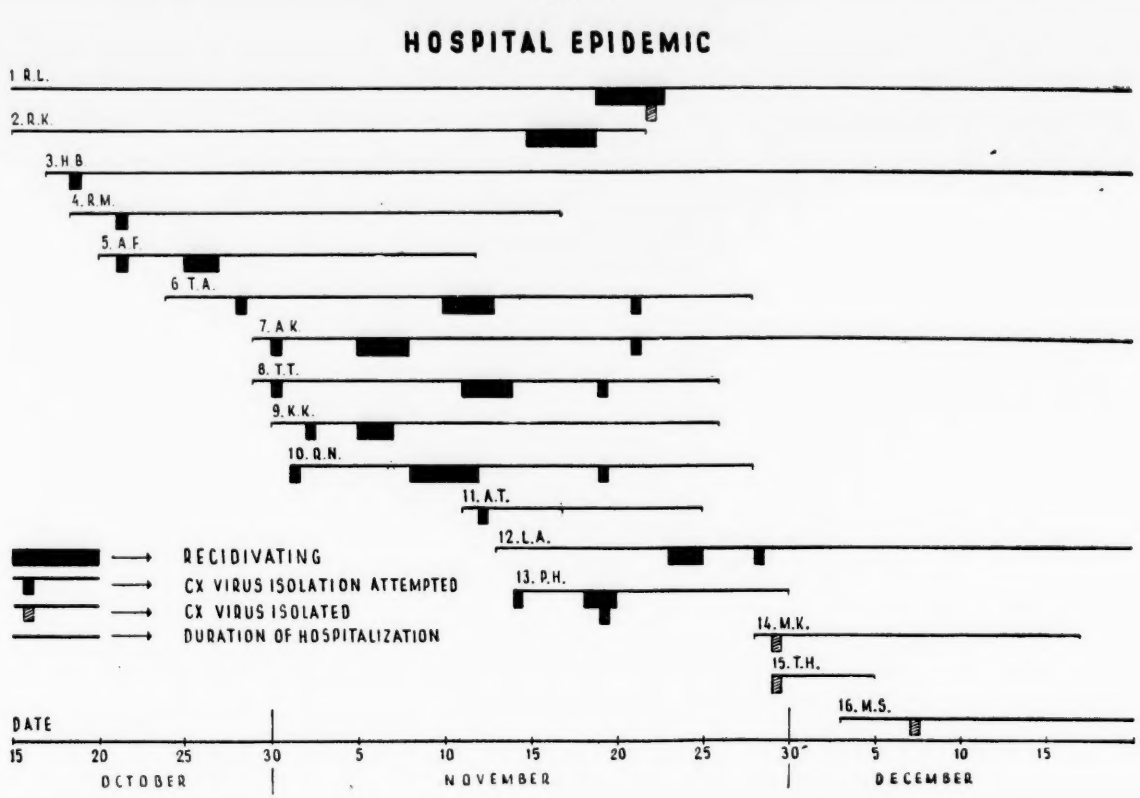
The total of diarrhoea cases examined was 187. Four Coxsackie

strains recovered from them were isolated twice and traced back to an individual patient. Three Coxsackie strains were isolated from mixtures of three stools each, all derived from diarrhoea patients. It proved impossible to re-isolate two of these virus strains as the stools included in the mixtures had been discarded by mistake. Neither could the third strain be re-isolated although two stools of the mixture were re-injected. In addition to the virus strains listed above, a Coxsackie strain was isolated from a mixture containing the stools of two diarrhoea patients and one poliomyelitis patient. One sample from a diarrhoea patient and that from the polio patient were re-injected but failed to induce paralysis in the mice. As all the four Coxsackie strains isolated from the mixtures represented virus types not handled in the laboratory at the time of the isolation, it is probable that they derived from the samples. The total of isolated viruses thus amounted to 7, possibly 8, which equals 4 per cent of all the diarrhoea cases studied.

*Hospital Epidemic.* — A mild hospital epidemic was observed in the Children's Clinic, Ward for Diarrhoea Patients in Helsinki, in November 1953. Patients that were already recovering re-contracted diarrhoea with concomitant fever. No further symptoms were observed. The disease lasted a few days only. The cause of the epidemic was not traced. Eleven out of the 15 patients of the ward had this relapse. Nine patients were examined for the Coxsackie virus either during or after the relapse. Sketch 1 shows the dates of the relapses and of the examination for Coxsackie virus. A Coxsackie virus of Type A 4 was isolated from Patient No. 1 (R.L.) during the relapse. This was no ordinary case of diarrhoea; the patient was under treatment for Coeliacia. However, the recidivation was similar to that revealed by the other patients of the ward. The patient in question had been examined for Coxsackie virus on admission two months previously. Neither the virus nor antibodies against the virus isolated later were found in the blood at that time. But 2½ months after the recidivation both neutralizing and complement-fixing antibodies were observed. No Coxsackie virus could be isolated from the remaining 8 patients who also had a relapse.

Soon after the epidemic described above a Coxsackie virus of Type A 4 was isolated from three patients treated in the same ward. However, in one patient (T.H.) the virus was recovered on the day of admission, in another (M.K.) it was found the day after admission. In addition, both had Coxsackie antibodies in the blood on entering the hospital. Hence it cannot be assumed that they contracted the infection in the ward. From the third patient the virus was isolated on the fourth day after admission. Since no blood sample was available from this patient it is difficult to say where

Sketch 1.


HOSPITAL EPIDEMIC
1. R.L.
2. R.K.
3. H.B.
4. R.M.
5. A.F.
6. T.A.
7. A.K.
8. T.T.
9. K.K.
10. R.N.
11. A.T.
12. L.A.
13. P.H.
14. M.K.
15. T.H.
16. M.S.
RECIDIVATING
CX VIRUS ISOLATION ATTEMPTED
CX VIRUS ISOLATED
DURATION OF HOSPITALIZATION
DATE
15 20 25 30 5 10 15 20 25 30 5 10 15
OCTOBER
NOVEMBER
DECEMBER

the infection originated. Two of these three patients had the diarrhoea in a very mild form. They had few diarrhoeal stools but were vomiting in addition. The third patient had a medium-severe diarrhoea of approx. 3 weeks' duration. None of the three patients had a sore throat, and one only had a slight fever.

*Discussion.* The Coxsackie virus was isolated from 4.4. per cent of the diarrhoea patients and from 1.1 per cent of the control material. The difference was 3.3. per cent (dispersion 1.6). The difference is significant.

During the hospital epidemic the Coxsackie virus was isolated from one patient; tests for antibodies showed that the patient had contracted the infection while in hospital. Thus it is possible that in this case the recidivation was due to Coxsackie infection. No Coxsackie virus could be isolated from another 8 patients who also experienced a relapse of the disease. However, it should be borne in mind that, apart from one case, the examination for virus was effected 5—13 days after the relapse. During diarrhoea, intestinal conditions are abnormal and it is possible that in such circumstances the virus disappears more rapidly than otherwise. This might account for the failure to isolate Coxsackie. The final inference must be, however, that there was no conclusive evidence to show that the reported epidemic was due to the Coxsackie virus.

*Minor Illness.* — To this group of diseases were referred the acute, relatively mild cases with indefinite symptoms that could not be attributed to a specific cause. As a rule these patients had fever and one or several of the following additional symptoms: headache, sore throat, muscular ache, or nausea. The presence in some of the patients of vesicles at the back of the mouth cannot be excluded but no particular investigation was made of this point. The group includes also a couple of cases with meningeal symptoms but no changes in the liquor. The total of this type of patient examined was 17. Three of them worked in the laboratory or were in close contact with a laboratory worker. Five were treated in hospital, the others stayed at home or worked on as usual in spite of their complaints. A total of 3 Coxsackie strains were isolated. One of them was found in a person employed in the laboratory, another in a member of her family, and the third from a sample submitted to the laboratory.

*Laboratory Infections.* — The laboratory caretaker and his wife began to look after the mice of the Virus laboratory March 1, 1953. They were responsible for feeding the animals infected with Coxsackie viruses and for cleaning their cages. On March 13 they both contracted, simultaneously, a minor illness, and their 2-year old son also fell ill after a few days.

A detailed report on the cases is given below.

*Mother*, 28, started a headache and chill shivers in the afternoon of March 13. She did not take her temperature but complained of feeling feverish. She had no sore throat. Cinema tickets had been bought for the evening but the patient felt too weak to go. She slept well during the night and felt perfectly well again in the morning.

Laboratory tests: A Coxsackie virus of Type A 10 was isolated from a faecal sample taken on March 22. Nineteen days after the onset of the disease both neutralizing and complement-fixing antibodies were found in the blood. (Page 52.)

*Son*, aged 2. March 16 his temperature suddenly rose to 38°C. The throat was slightly sore. The patient was still unwell the following day and refused to go out to play with other children. By March 18 the symptoms had completely subsided.

Laboratory tests: A Coxsackie virus of type A 10 was isolated from a faecal sample taken on March 22. No examination for antibodies was made.

*Father*, 29, did not feel well on the morning of March 13. His throat was slightly sore. He felt tired and complained of a rise in temperature although it was not taken. In addition he had indefinite aches in the muscles. He felt as if he had had a tight belt on just above the waist. The head ached around the eyes, in the temple and at the back of the head. In spite of his condition the patient did his work. The muscular pain, fatigue and headache persisted during the following days. From time to time he had a feeling of having been cut with a knife at the back just above the waist. The complaints persisted for approximately a week.

Laboratory tests: A faecal sample of March 22 was examined for Coxsackie virus but the result was negative. Nine days after the onset of the disease both neutralizing and complement-fixing antibodies against type A 10 were found in the blood. (Page 52.)

Unfortunately the cases were only reported afterwards when it was too late to examine the patients. Hence it is not known with certainty whether the father and son in connection with their sore throats also had vesicles at the back of the mouth. The patients themselves noticed no such symptom. The Coxsackie virus could only be isolated from mother and son. But if their disease was induced by the Coxsackie virus it can be assumed that the father's illness too arose from the same cause. Provided this was the case, it is worth noting that although the disease presumably can be referred back to a Group A virus the father had symptoms highly suggestive

of epidemic myalgia. This would contradict the generally held view that epidemic myalgia is induced by viruses of Group B only.

A virus of type A 4 was isolated from other samples submitted to the laboratory. The virus-containing sample derived from a boy of 16, of Loimaa. The patient had high fever and ear-ache, nausea and vomiting. The nausea passed over in three days but fever persisted for a week. The general practitioner at Loimaa had simultaneously had under treatment at least ten patients with symptoms like those described above; thus a kind of epidemic seems to have been in question. But no samples were submitted for examination from the rest of the patients. About the same time, 5 cases of paralytic poliomyelitis were diagnosed at Loimaa.

## ANTIBODIES IN THE SERUM

The virus strains used as antigens in both the neutralization and complement fixation test were those employed in typing (p. 36).

### COMPARISON OF NEUTRALIZING AND COMPLEMENT-FIXING ANTIBODIES

*The Patients.*

In order to facilitate the comparison of complement-fixing titres efforts were made to titrate all the sera of an individual patient simultaneously.

Apart from one (Case 7), the Coxsackie virus was isolated from all the patients examined. However, even in the exceptional case there was every reason to suspect Coxsackie infection (p. 52).

The sera of 13 patients were tested for neutralizing antibodies specific to the virus found in each case. Two sera were not tested for complement-fixing antibodies as the titre of the virus isolated from these patients was too low to permit the preparation of antigen effective for the complement-fixation reaction. Where the sera were adequate they were also tested for complement-fixing antibodies against some heterologous Coxsackie types. The results are shown in Table 7.

Every patient was found to have neutralizing antibodies against their own virus type. Only one of the sera tested for complement-fixing antibodies failed to show them. This was a child of 11 months

TABLE 11

ANTIBODIES IN PATIENTS

| No. | Patient | Days after onset of disease | Type isolated | Homol. antibodies | | Heterol c.f. antibodies | | |
|---|---|---|---|---|---|---|---|---|
| | | | | neutr. | c.f. | A 4 | A 5 | A 10 |
| 1 | L. K. 5 yrs | 7 | B K | 4 | | 8 | 4 | — |
| | | 18 | | 4 | | 8 | 4 | — |
| | | ? | | | | 16 | 4 | — |
| 2 | L. E. 12 yrs | 4 | E | 2 | | 8 | 16 | 32 |
| | | 11 | | 2 | | 8 | 16 | 32 |
| | | 27 | | 2 | | 8 | 16 | 32 |
| 3 | I. L. 7 yrs | 10 | A 4 | 2 | — | | 4 | 4 |
| | | 195 | | >3 | 16 | | 8 | 16 |
| 4 | P. P. 3 yrs | 6 | A R | 3 | — | 16 | — | — |
| | | 17 | | >4 | 32 | 32 | — | — |
| * 5 | P. G. 27 yrs | 9 | A 10 | <3 | — | | | |
| | | 19 | | <3 | — | | | |
| | | 30 | | 3 | 16 | | | |
| | | 325 | | 3 | 4 | | | |
| | | 417 | | 3 | — | | | |
| * 6 | A. L. 31 yrs | 2 | A 10 | — | — | — | 32 | |
| | | 7 | | 3 | — | — | 32 | |
| | | 14 | | 3 | 16 | 32 | 32 | |
| | | 32 | | 3 | 16 | 16 | 32 | |
| | | 75 | | 3 | 16 | 32 | 32 | |
| | | 240 | | 2 | | | | |
| * 7 | A. T. 29 yrs | 7 | A 10 | 3 | 8 | — | — | |
| | | 19 | | 3 | 8 | — | 4 | |
| | | 29 | | 3 | 16 | — | 8 | |
| | | 63 | | 3 | 8 | | | |
| * 8 | E. T. 28 yrs | 9 | A 10 | 2 | *** Si | Si | Si | |
| | | 19 | | 2 | 16 | | 8 | |
| | | 37 | | 2 | 8 | 8 | — | |
| | | 63 | | 2 | S e | | | |
| 9 | R. L. 2 yrs | ** | A 4 | — | — | | | |
| | | 73 | | >2 | 8 | | | |
| 10 | M. K. 1 yrs | 60 days | A 4 | >2 | 32 | | | |
| | | later | | >2 | 8 | | | |
| 11 | T. H. | 8 | A 4 | >4 | 32 | | | |
| | 4 mths | 87 | | >4 | 32 | | | |
| 12 | I. M. 4 yrs | 17 | A 5 | — | — | | | |
| | | 44 | | 2 | — | | | |
| 13 | E. S. 34 yrs | 33 | A 10 | 3 | 8 | | | |
| | | 153 | | >3 | 4 | | | |
| | | 240 | | >3 | 16 | | | |

* Laboratory infections
** Before onset of disease
*** Si = Seruminhibition.

whose neutralizing antibodies too seem to have increased slowly and never reached more than a low titre (Case 12).

*Appearance of Antibodies.* — *Neutralizing antibodies* were encountered as soon as on the 4th day after the onset of the disease (Case 2), but not as early as on the 2nd day (Case 6). One patient failed to show these antibodies as late as 17 days after the onset of the disease (Case 12). Two children under 10 (Cases 3, 4) were found to show an increase in antibodies as late as a week after the onset of the disease. It is possible that a slight increase might have been observable with other patients too had the method of investigation been more accurate.

*Complement-fixing antibodies* develop later than the neutralizing antibodies. As a rule they were not encountered before 10 days had elapsed from the onset of the disease (Cases 3, 4, 5, 6). One patient (Case 7), however, showed these antibodies in one week. Another (Case 6) showed them after 14 days and yet another (Case 4) after 17 days. In one case (Case 5), complementfixing antibodies were still not found after 19 days, but were stated after a further 10 days.

*Persistence of Antibodies.* — With a single patient (Case 5) the antibodies were followed up for over a year. This person showed *neutralizing antibodies* as late as 14 months after Coxsackie infection. The blood samples of two patients (Cases 6, 13) were received 8 months after the onset of the disease; both samples still contained neutralizing antibodies. In one of them (Case 6), however, the titre had apparently fallen somewhat.

*Complement-fixing antibodies* persist after 6 months (Cases 3, 5, 13). One patient (Case 5) showed them after a year although the titre had dropped; two months later they had disappeared. With another patient (Case 13) the antibody titre dropped at the end of six months but seems to have risen again later. This may, of course, be the result of an error in the laboratory, but it is also possible that the person in question was re-infected by another Coxsackie type which induced an »anamnestic» increase in antibodies.

*Heterologous Complement-fixing Antibodies.* — The sera of six patients were also tested for complement-fixing antibodies against certain heterologous Coxsackie types. An increase in the antibodies against one heterologous type was observed in two patients (cases 4, and 6), against two heterologous types in one patient (case 3).

*The Healthy Subjects*

*Material.* — The examination covered the sera taken in February 1953 from healthy conscripts, a couple of days after the men had reported at the garrison. The conscripts came from different parts of Finland, the majority from South Finland. Their ages ranged from 19—25 years; the majority were 19 years old. Neutralizing antibodies against A 10 were determined from healthy medical students of 22—25 from whom the blood samples were taken in the autumn of 1951.

*Complement-fixing Antibodies. — Incidence.* 50—75 sera were tested for complement-fixing antibodies against seven types of Coxsackie virus. The results are shown in Table 12. A 1 antibodies are in an overwhelming majority. They were found in 90 per cent

TABLE 12

THE OCCURRENCE OF COMPLEMENT-FIXING ANTIBODIES

| Type | No. examined | No. posit. | Per cent | Dispersion* |
|---|---|---|---|---|
| A 1 | 75 | 68 | 91 | 3 |
| A 2 | 75 | 7 | 9 | 3 |
| A 3 | 75 | 14 | 19 | 4 |
| A 4 | 50 | 23 | 46 | 7 |
| A 5 | 75 | 28 | 37 | 6 |
| A 8 | 67 | 16 | 24 | 5 |
| A 10 | 75 | 5 | 7 | 3 |

* Dispersion s is the measure of the accuracy of the observed percentage p, so that the confidence limits corresponding to the 95 % probability are $p \pm 2\,s$ and the confidence limits corresponding to the 99.7 % probability are $p \pm 3\,s$.

TABLE 13

THE TITRES OF COMPLEMENT-FIXING ANTIBODIES

| Type | Titre * | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | < 4 | 4 | 8 | 16 | 32 | 64 | 128 | Total |
| A 1 | 9 | 23 | 23 | 26 | 2 | 1 | 1 | 75 |
| A 2 | 68 | 2 | 1 | 3 | 1 | — | — | 75 |
| A 3 | 61 | 7 | 5 | 2 | — | — | — | 75 |
| A 4 | 27 | 5 | 9 | 8 | 1 | — | — | 50 |
| A 5 | 47 | 10 | 15 | 3 | — | — | — | 75 |
| A 8 | 59 | 7 | 7 | 2 | — | — | — | 67 |
| A 10 | 70 | 0 | 3 | — | — | — | — | 75 |

* The numerals in every column express the numbers of sera. — The titre is expressed in inverse ratio of the original dilution of the serum.
Not till tabell 12

of the sera. Antibodies against all the other types were present in under 50 per cent of the sera. It is curious to see that the A 10 antibodies were the least common — found in only 7 per cent of the sera — although several viruses of this type had been isolated (p. 38).

The occurrence of the different types of complement-fixing antibodies was in no way interconnected.

The *titres* as a rule were low, 1/4—1/6 (Table 13). No appreciable differences were observed between the different types on this point.

*Neutralizing Antibodies. — Incidence.* Neutralizing antibodies against eight Coxsackie types were examined from 15—25 sera (Table 14). Antibodies were found against each type. All the

TABLE 14

THE OCCURRENCE OF NEUTRALIZING ANTIBODIES

| Type | No. examined | No. posit. | Per cent | Dispersion |
|---|---|---|---|---|
| A 1 | 20 | 15 | 75 | 10 |
| A 2 | 21 | 19 | 90 | 6 |
| A 3 | 17 | 12 | 71 | 11 |
| A 4 | 19 | 18 | 95 | 5 |
| A 5 | 28 | 24 | 86 | 7 |
| A 0 | 21 | 11 | 52 | 11 |
| A 10 | 20 | 16 | 80 | 9 |
| B 1 | 15 | 9 | 60 | 13 |

antibodies were established in over half the number of persons examined. A 4 antibodies had the highest incidence; only from a single serum tested were they missing. It should be noted that the sera were not taken at random for the naturalization tests; they were selected so as to include a somewhat higher proportion of sera containing complement-fixing antibodies than is warranted by their frequency in an unselected material. It is possible that the incidence of neutralizing antibodies in unselected sera would have been slightly lower than it actually was here.

Some sera (8, 13, 15, 20, 21, 28, 29, 50, 67, 68), however, were tested for antibodies against several types. From this it may be concluded that none of the antibodies were interconnected in their occurrence. A serum may contain antibodies against any one of the types and yet against none of the remaining types tested.

TABLE

COMPARISON OF NEUTRALIZING

| A 1 | | | A 2 | | | A 3 | | | A 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | C.f. | Neutr. | No. | C.f. | Neutr. | No. | C.f. | Neutı. | No. | C.f. | Neutr. |
| 1 | — | — | 7 | 16 | > 3 | 13 | 8 | > 3 | 75 | — | > 2 |
| 3 | 8 | 3 | 10 | 4 | 3 | 15 | 16 | > 3 | 78 | — | > 2 |
| 4 | 8 | 3 | 15 | 8 | > 3 | 20 | 8 | 2 | 79 | — | > 2 |
| 6 | 16 | 2 | 17 | — | — | 21 | — | — | 80 | — | > 2 |
| 12 | 8 | — | 21 | — | — | 24 | — | — | 81 | — | > 2 |
| 13 | 8 | 2 | 24 | — | 3 | 28 | — | 3 | 83 | — | — |
| 14 | 8 | 2 | 26 | — | > 3 | 29 | — | — | 84 | — | > 2 |
| 17 | 8 | > 3 | 30 | — | 3 | 30 | — | 4 | 85 | 8 | > 2 |
| 18 | 8 | 2 | 31 | — | > 4 | 31 | — | 2 | 86 | — | > 2 |
| 20 | 32 | — | 34 | — | 4 | 34 | — | > 3 | 88 | 8 | > 2 |
| 28 | 16 | — | 37 | — | 4 | 35 | — | — | 90 | 8 | > 2 |
| 29 | 8 | 2 | 68 | 4 | 4 | 38 | — | 3 | 92 | 16 | > 2 |
| 32 | 32 | 2 | 8 | — | > 2 | 39 | — | 3 | 93 | — | > 2 |
| 39 | 8 | — | 9 | — | > 2 | 40 | — | 2 | 94 | 8 | > 2 |
| 54 | — | 2 | 14 | — | > 2 | 50 | 4 | > 3 | 95 | 16 | > 2 |
| 8 | 4 | > 2 | 18 | — | > 2 | 52 | 8 | > 3 | 96 | 16 | > 2 |
| 9 | 8 | > 2 | 22 | — | > 2 | 67 | — | — | 98 | 8 | > 2 |
| 11 | 16 | > 2 | 23 | — | > 2 | | | | 101 | — | > 2 |
| 15 | 16 | > 2 | 35 | — | > 2 | | | | 104 | — | > 2 |
| 25 | 32 | > 2 | 38 | — | > 2 | | | | | | |
| | | | 70 | — | > 2 | | | | | | |

**The complement fixing titres are expressed in inverse ratios of the dilution of the serum. The neutralizing titres are expressed as a negative logarithm of the dilution of the virus.**

*Titres.* As the titration of neutralizing antibodies consumes a great deal of mice only a few sera were tested. These few experiments hardly permit conclusions, but it seems as if the titres of A 1 antibodies were lower than those of the other types (Table 15).

*Comparison of Complement-fixing and Neutralizing Antibodies.* — The occurrence of neutralizing antibodies was found to exceed that of the complement-fixing. An exception was Type A 1 which had more complement-fixing than neutralizing antibodies.

The ratios of occurrence of complement-fixing and neutralizing antibodies appear to differ somewhat with the different types (cf. Types A 2, A 5).

15

AND COMPLEMENT-FIXING ANTIBODIES

| A 5 | | | A 8 | | | A 10 | | | B 1 | |
|---|---|---|---|---|---|---|---|---|---|---|
| N:o | C.f. | Neutr. | N:o | C.f. | Neutr. | No. | C.f. | Neutr. | N:o | Neutr. |
| 1 | — | — | 20 | 4 | > 3 | d 48 | — | — | 77 | — |
| 2 | — | > 3 | 21 | — | — | » 64 | — | — | 78 | > 1 |
| 3 | — | 2 | 41 | — | — | » 65 | — | — | 80 | > 1 |
| 4 | 4 | 2 | 42 | — | — | » 66 | — | 2 | 82 | > 1 |
| 7 | 8 | 2 | 43 | — | — | » 67 | — | 3 | 83 | > 1 |
| 8 | — | — | 47 | — | 3 | » 68 | — | 2 | 84 | > 1 |
| 10 | 16 | > 3 | 48 | — | — | » 69 | — | 2 | 85 | — |
| 12 | — | > 3 | 49 | 8 | > 3 | » 70 | — | — | 86 | — |
| 13 | 8 | > 3 | 50 | 4 | 4 | » 71 | — | 2 | 88 | — |
| 28 | 8 | 2 | 51 | — | — | » 72 | 4 | 2 | 90 | — |
| 29 | 8 | 4 | 52 | 8 | 3 | » 73 | 4 | 3 | 91 | > 1 |
| 36 | 8 | 2 | 53 | — | — | » 74 | 8 | > 3 | 92 | > 1 |
| 46 | 8 | 4 | 54 | — | 2 | » 75 | 16 | > 4 | 93 | — |
| 48 | — | 3 | 64 | 8 | — | » 33 | — | > 2 | 94 | > 1 |
| 50 | 8 | > 2 | 65 | — | > 3 | » 49 | 8 | > 2 | 95 | > 1 |
| 51 | — | 3 | 66 | — | — | » 53 | — | > 2 | | |
| 53 | — | > 3 | 68 | — | — | » 54 | — | > 2 | | |
| 54 | — | 3 | 69 | 4 | 3 | » 56 | 16 | > 2 | | |
| 58 | — | 4 | 13 | 8 | > 2 | » 57 | — | > 2 | | |
| 59 | — | 2 | 15 | 8 | > 2 | » 60 | — | > 2 | | |
| 63 | 8 | 2 | 46 | — | > 2 | | | | | |
| 64 | 4 | 3 | | | | | | | | |
| 67 | 4 | 4 | | | | | | | | |
| 68 | 8 | > 2 | | | | | | | | |
| 69 | — | — | | | | | | | | |
| 70 | 8 | > 3 | | | | | | | | |
| 72 | — | — | | | | | | | | |
| 74 | — | > 2 | | | | | | | | |

Table 15 compares the complement-fixing and neutralizing antibodies of each serum. Apart from Type A 1, it seems that sera containing complement-fixing antibodies also reveal neutralizing antibodies against the same type. One serum (64), however, has complement-fixing A 8 antibodies although the result of the neutralization test against this type was negative. As a matter of fact, the serum in question did contain neutralizing A 8 antibodies also, but as the titre was low, only 1, it was counted as negative.

The correlation between the *titres* of complement-fixing and neutralizing antibodies is very slight.

*Discussion.* — As in earlier investigations (54), it was found that complement-fixing antibodies develop later and disappear sooner than neutralizing antibodies. This explains the contention that more neutralizing than complement-fixing antibodies are found in healthy subjets.

It was found above that the ratio of occurrence of complement-fixing and neutralizing antibodies did not seem identical with different types. This is understandable if it is borne in mind that complement-fixing antibodies are evidence of a recent infection while neutralizing antibodies also indicate an infection contracted earlier. Hence it may be assumed that complement-fixing antibodies against the last current Coxsackie type are relatively most common. Another possibility is a variation in the persistence of the different types of complement-fixing and neutralizing antibodies. Type A 1 differs from the others in that the complement-fixing antibodies against it are more abundant than the neutralizing. This might be because the antibodies against this type have a completely different duration, the complement-fixing antibodies persisting longer than the neutralizing. Another explanation is that A 1 antigen may give unspecific reactions in the complement-fixation test.

It was concluded earlier (p. 27) that the increase in heterologous complement-fixing antibodies could have an anamnestic origin. This is supported by the finding that sera containing complement-fixing antibodies regularly revealed neutralizing antibodies against the same type (with the exception of Type A 1). The occurrence of complement-fixing antibodies of different types seems to be in no way interconnected. This indicates that Coxsackie types have no common antigen factor that appears regularly and can be shown only by complement fixation method. If the above assumption — that the increase in heterologous antibodies is an anamnestic reaction — is tenable, there is every justification for drawing conclusions on the incidence of a certain virus type from the incidence of complement-fixing antibodies against this type in a healthy population.

### COMPLEMENT-FIXING ANTIBODIES IN THE DIFFERENT AGE GROUPS

*Sera.* — Samples of umbilical cord blood were obtained from Helsinki University Women's Clinic in the autumn of 1953.

Blood samples from 0—1 year old infants were obtained from healthy children in the »Folkhälsan» Nursing Home during the late winter of 1952

and from patients of Helsinki University Children's Clinic during the spring of 1954. (No cases with a diagnosis of meningitis, poliomyelitis, gastroenteritis or sore throat were included in the titrations.)

Blood samples from Children 1—16 years old were obtained from the Children's Clinic (see above) and from healthy children in the »Sofianlehto» Home during the spring of 1954.

Blood samples from the 19—50 age group were obtained from the Finnish Red Cross Blood Bank during the autumn of 1953.

The blood samples of persons over 50 were obtained from the Finnish Red Cross Blood Bank during the autumn of 1953 and from Helsinki Communal Home during the spring of 1954.

The sera are relatively evenly distributed, in the first group over the different months and in the other groups over the different years of age.

Children's sera were always titrated in combination with the sera of adults, never alone.

*Incidence of Antibodies.* — Tests for antibodies against three Coxsackie types, A 1, A 5 and A 10, were made for the different age groups (Table 16). It was found that the antibodies are relatively abundant at birth. Their incidence in the umbilical cord blood samples is at least equal to that in the blood of the older children and of adults. In addition, the titres are higher on an average in this group than in the other groups. Soon, however, there is sharp fall in antibodies. Children under 1 year have the least antibodies. Antibodies increase gradually and reach their maximum in the 7—16 age group. Subsequently the antibodies against A 1 seem to decrease.

TABLE 16

COMPLEMENT-FIXING ANTIBODIES IN DIFFERENT AGE GROUPS

| | A 1 | | | | A 10 | | | | A 5 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. examined | No. posit. | Per cent | Dispersion | No. examined | No. posit. | Per cent | Dispersion | No. examined | No. posit. | Per cent | Dispersion | |
| Umbilical cords .... | 25 | 24 | 96 | 4 | 21 | 5 | 24 | 9 | 21 | 13 | 62 | 11 | Autumn-53 |
| 0—11 months | 36 | 7 | 19 | 7 | 42 | 1 | 2 | 2 | 40 | 1 | 2 | 2 | Spring-54 |
| 1—6 years .. | 37 | 26 | 70 | 8 | 37 | 5 | 14 | 6 | 38 | 10 | 26 | 7 | Spring-54 |
| 7—16 years.. | 30 | 27 | 90 | 5 | 30 | 6 | 20 | 7 | 32 | 14 | 44 | 9 | Spring-54 |
| 19—50 years | 59 | 38 | 64 | 6 | 58 | 13 | 22 | 5 | 57 | 12 | 21 | 5 | Autumn-52 |
| Over 50 years | 43 | 19 | 44 | 8 | 35 | 6 | 17 | 6 | 36 | 12 | 33 | 8 | Autumn-52 and Spring-54 |

*Discussion.* — A 1 and A 5 antibodies are at their maximum in the 7—16 age group. It is interesting to note that in the United States considerably less A 4 complement-fixing antibodies were found in the 10—14 age group than in the 7—9 age group although the neutralizing antibodies in both groups were roughly the same in occurrence (74). The explanation advanced was that a large proportion of the 10—14 age group had neutralizing antibodies as a result of earlier Coxsackie infections while complement-fixing antibodies had already begun to disappear. The neutralizing antibodies protected them against a new Coxsackie infection and thus also inhibited the formation of new complement-fixing antibodies.

### THE REGIONAL INCIDENCE OF COMPLEMENT-FIXING ANTIBODIES IN FINLAND

*Material.* — The majority of the sera were obtained from military conscripts during the late winter of 1954 (for details see p. 54). In addition, a few sera were obtained from healthy expectant mothers admitted to Rovaniemi Maternity Hospital during the autumn of 1953.

*Incidence.* — The sera of 227 conscripts were tested against three Coxsackie types, A 1, A 5 and A 10. A study of the incidence of antibodies by zones from *south to north* revealed no significant differences between the zones. As only a few of the conscripts came from North Finland, an additional 26 sera taken from expectant mothers in Rovaniemi Maternity Hospital were examined. The incidence of antibodies in these 26 samples was found to comply roughly with that in the sera of the 19—50 age group collected in South Finland.

TABLE 17

COMPLEMENT-FIXING ANTIBODIES IN WEST AND EAST FINLAND

| | A 1 | | | | A 5 | | | | A 10 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. examined | No. posit. | Per cent | Dispersion | No. examined | No. posit. | Per cent | Dispersion | No. examined | No. posit. | Per cent | Dispersion |
| West Finland | 106 | 82 | 77 | 4 | 116 | 30 | 26 | 4 | 116 | 12 | 10 | 3 |
| East Finland | 99 | 76 | 77 | 4 | 104 | 38 | 37 | 5 | 104 | 22 | 21 | 4 |
| Difference | | | 0 | 6 | | | 11 | 6 | | | 11 | 5 |

The incidence of the three kinds of antibodies in *West and East Finland* was also investigated (Table 17). A 1 antibodies were found to have the same incidence in both regions. A 5 and A 10 antibodies, however, were found slightly more frequently in samples from East Finland. For A 5 the difference was 11 per cent, dispersion 6; the difference is not significant. For A 10 the difference was also 11 per cent, but dispersion 5; thus this difference is significant.

TABLE 18

COMPLEMENT-FIXING ANTIBODIES IN URBAN- AND RURAL DWELLERS

| | A 1 | | | | A 5 | | | | A 10 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. examined | No. posit. | Per cent | Dispersion | No. examined | No. posit. | Per cent | Dispersion | No. examined | No. posit. | Per cent | Dispersion |
| Urban dwellers | 94 | 80 | 85 | 4 | 103 | 36 | 35 | 5 | 103 | 16 | 16 | ±4 |
| Rural dwellers | 115 | 79 | 69 | 4 | 124 | 32 | 26 | 4 | 124 | 18 | 15 | ±3 |
| Difference | | | 16 | 6 | | | 9 | 6 | | | 1 | ±5 |

In addition, the conscripts were classified into *urban and rural dwellers* (Table 18). The urban dwellers were found to reveal a slightly higher incidence of A 1 and A 5 antibodies, but no difference in A 10 antibodies. 16 per cent more urban dwellers had A 1 antibodies compared with rural dwellers. The dispersion is 6; hence the difference is significant. For A 5 the difference was 9 per cent, dispersion 6. This difference is not significant.

# DISCUSSION

The survey of the literature revealed that the pathogenic significance of the Coxsackie group is a problem still to be resolved. The aim of the present investigation is to contribute to the solution by means of epidemiologic investigations. The incidence of Coxsackie viruses in the different disease groups has been compared with its incidence in the control group. In addition Coxsackie antibodies have been studied in persons with Coxsackie infection and in healthy population.

The literature contains numerous reports on Coxsackie findings in connection with *paralytic poliomyelitis*. The absence in most cases of a control material of normal subjects makes it impossible to be certain whether the incidence of Coxsackie viruses is higher in connection with this disease than it is in healthy subjects. In the present work it was possible to isolate the Coxsackie virus from 2 per cent of the cases of paralytic poliomyelitis. The difference from the control material is only 1 per cent (dispersion 2), i.e. not significant. This suggests that the incidence of Coxsackie viruses is no higher in connection with paralytic poliomyelitis than it is in healthy subjects.

Several authors have reported isolates of Coxsackie viruses from *cases of meningitis and encephalitis*. In Sweden, the virus was recovered more often from meningitis patients than from the control material. Moreover slight meningeal symptoms have been observed in two persons who had contracted laboratory infections. This suggests that some Coxsackie types at least may induce meningitis in man. In the present investigation the Coxsackie virus was isolated from 7 per cent of the cases of meningitis and encephalitis. The difference from the control material is 6 per cent (dispersion 2.5), which is significant. These results support the

view that Coxsackie viruses may induce meningitis and encephalitis in man.

During some *herpangina* epidemics in the United States it has been possible to isolate the Coxsackie virus from almost all the patients, and one person with a laboratory infection revealed in the posterior part of the pharynx a vesicle very similar to the type found in herpangina patients. These investigations suggest that herpangina may be a specific disease induced by Coxsackie viruses. In Sweden, however, Coxsackie infection was found in only 4 out of 11 sporadic cases of herpangina. This finding makes it questionable whether Coxsackie virus is the only agent of herpangina.

The present investigation reports on two laboratory infections in which the patients showed symptoms of herpangina — support for the idea that Coxsackie viruses may induce this disease. Yet the virus could only be isolated from 31 per cent of the cases of herpangina. This again concurs with the investigations carried out in Sweden and supports the assumption that the Coxsackie virus is not the only agent to induce herpangina.

There are several instances of Coxsackie isolations from patients suffering from a *minor illness*. In connection with some laboratory infections, moreover, minor illnesses have been stated in which no vesicles were observed in the pharynx. It seems, therefore, that the Coxsackie group may induce a disease of this type. The present investigation also reports on two laboratory infections resulting in a minor illness without oral vesicles according to the patients themselves. This supports the view that the Coxsackie virus can induce a minor illness.

Reports in the literature point out that Coxsackie viruses have sometimes been isolated from cases of disease in which *diarrhoea* was one of the symptoms. However, the occurrence of Coxsackie viruses in conjunction with infantile diarrhoea has been reported from only one investigation, and that with a limited material. In the present investigation the Coxsackie virus was isolated from 4 per cent of the diarrhoea patients. The difference from the control material is 3.3 per cent (dispersion 1.6), which is significant.

It was established earlier that neutralizing *antibodies* against the viruses of Group B *develop* sooner and persist longer than

complement-fixing antibodies. In the present investigation the antibodies against the viruses of Group A showed a similar development. Another earlier observation was that an increase in heterologous complement-fixing Coxsackie antibodies follows a Coxsackie infection. The same phenomenon was seen in the present work too.

Investigations in other countries suggest that neutralizing *antibodies* against all Coxsackie types are present in abundance in the *healthy population*. In many cases, however, the findings have been based on a very limited material. In Europe, only Germany and Finland have studied Coxsackie antibodies. The incidence of complement-fixing Coxsackie antibodies in the healthy population has been little investigated at all. The present investigation established neutralizing Coxsackie antibodies against all the Coxsackie types studied in over half the number of the persons examined. But the incidence of complement-fixing antibodies was much lower. This was only to be expected, since neutralizing antibodies persist much longer than complement-fixing antibodies. An exception is type A 1, against which there were more complement-fixing than neutralizing antibodies.

The abundant presence of antibodies in the population and the fact that the virus could be isolated from 1 per cent of the control material are an indication of the high incidence of Coxsackie viruses. In spite of this, Coxsackie viruses could be isolated from relatively few cases of the disease groups investigated, herpangina excepted. Herpangina, again, in its typical and relatively severe form, is no widespread disease according to the general practitioners, at least not common enough for the majority of people to have had it once or even several times during their lives. In laboratory infections the symptoms have usually been quite mild. Four laboratory infections were reported in the present investigation. Three resulted in a very mild disease, so slight that it would hardly have attracted attention at all had not a careful check been kept on all illnesses because of possible Coxsackie infection. All this seems to suggest that a large part of Coxsackie infections are subclinical or induce illness so slight that the patient is not prompted to consult a doctor.

## SUMMARY AND CONCLUSIONS

*Isolation of Viruses.* — A total of 657 faecal samples, all from different persons, were examined. 36 Coxsackie strains were isolated from them; six, however. were evidently derived from the laboratory. Four of these were isolated from persons working in the Virus Laboratory or from their close relatives. Another two were probably not in the samples at all originally but entered them via the laboratory during the tests. Thus 30 Coxsackie strains were isolated from the samples submitted to the laboratory, i.e. 5 per cent of the cases examined.

The following data apply only to the samples submitted to the laboratory.

*Types.* — Twelve of the isolated viruses were of type A 4, ten of type A 10, one of type A 5, one of type B 1 and one of type B 3. In addition, the strains found included five that could not be typed. Three of them were mutually identical, marked AR. The remaining two strains were mutually different, marked with E and BK.

*Seasonal Incidence.* — Coxsackie virus was isolated from 3 per cent of the samples taken in the first half of the year, from 5 per cent of those taken in the latter 6 months. In samples taken between the beginning of the year and June, however, only one Coxsackie strain was isolated.

*Incidence by Age Groups.* — Coxsackie virus was isolated from 5 per cent of the subjects under 10 and 2 per cent of those over 10 years of age.

*Incidence by Diseases and Control Cases.* — Coxsackie virus was isolated from 32 per cent of the cases of herpangina (types A 4, A 10 and AR), 7 per cent of the cases of meningitis and encephalitis (types A 4, A 5, A 10, AR, E and BK), 4 per cent of the cases of

gastroenteritis (types A 4 and B 1) and 2 per cent of the cases of paralytic poliomyelitis (type A 10). Fourteen patients with a minor illness yielded a single Coxsackie isolate (type A 4). In the control material, the virus was isolated from 1 per cent (types A 4 and A 10).

*Laboratory Infections.* — A Coxsackie virus of type A 10 was isolated from 3 laboratory workers and one person in their families. All these persons felt slightly ill just before or when the virus was isolated. Two of them had symptoms, in one case very mild, of herpangina. The other two patients, mother and son, both suffered from a minor illness. The father of the family too, who worked in the laboratory, fell ill at the same time as the other members but showed symptoms of epidemic myalgia. In his case, however, the result of the virus test was negative.

*Antibodies in the Patients.* — 13 patients from whom Coxsackie virus had been isolated were subjected to examination; all showed homologous neutralizing antibodies. Tests for homologous complement-fixing antibodies were made with 11 patients. They were established in the sera of all but one of the patients.

Neutralizing antibodies were found 4 but not as early as 2 days after the onset of the disease. Complement-fixing antibodies developed later. As a rule they were not encountered during the first 10 days.

Three out of six patients also showed an increase in heterologous complement-fixing antibodies after a Coxsackie infection.

One patient could be followed up for over a year. 14 months after the Coxsackie infection this patient still revealed the presence of neutralizing antibodies, but no complement-fixing antibodies.

*Antibodies in Healthy Subjects.* — A 1, A 2, A 3, A 4, A 5, A 8 and A 10 complement-fixing antibodies were tested in 50—75 sera and neutralizing antibodies in 17—25 sera, as were neutralizing antibodies against B 1 in 15 sera. Both antibodies were found to exist against each type. Neutralizing antibodies against each type were established in over half the number of the persons examined. The incidence of complementfixing antibodies was considerably lower. An exception is type A 1, against which there were more complement-fixing than neutralizing antibodies.

The complement-fixing antibodies of the different Coxsackie

types did not seem to be interrelated. The ratio neutralizing: complement-fixing antibodies of the different types was not constant.

Apart from Type A 1, it seems to be the rule that sera containing complement-fixing antibodies also have neutralizing antibodies against the same type.

*Antibodies by Age Groups.* — A 1, A 5 and A 10 complement-fixing antibodies were tested in sera from different age groups. In umbilical cord blood samples the incidence of antibodies was found to be at least equal to that in the sera of older children and adults. Infants under 1 year have less antibodies, but they increase gradually to a maximum at the age of 7—16. Persons over 50, again, have less antibodies than the 7—16 age group.

*The Regional Incidence of Complementfixing Antibodies in Finland.* — Tests were made to determine the presence of complementfixing antibodies against types A 1, A 5 and A 10 in sera from different parts of Finland. No differences were found between the various zones from north to south, but in Eastern Finland the incidence of A 5 and A 10 antibodies was slightly higher than in Western Finland.

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It is obvious that Coxsackie viruses may be pathogenic to man. The best indication of this is provided by the laboratory infections experienced in various countries. It may be considered highly probable that Coxsackie viruses induce in man herpangina, epidemic myalgia, minor illness and meningeal symptoms. But the virus could be isolated from healthy subjects too and its antibodies seem to occur in abundance in the normal population.

In the present investigation, Coxsackie virus could be isolated from 1 per cent of the control material and healthy subjects revealed a high incidence of its antibodies. In spite of this, relatively few viruses could be isolated from the disease groups examined. It is also worthy of note that the laboratory infections were attended by very mild symptoms. All this speaks in favour of the majority of the Coxsackie infections being subclinical or inducing only a very mild minor illness.

# APPENDIX

COXSACKIE VIRUS ISOLATIONS

| No | Patient | Age | Sex | Date | Type | Diagnosis | Antibodies in the serum | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Neutr. | C. f. |
| 1 | J. E. | 5 yrs | M | 20. 9.50 | A 10 | Meningitis | | |
| 4 | H. T. | 27 yrs | F | 26. 8.50 | A 10 | Meningitis | + | + |
| 8 | J. K. | 34 yrs | M | 9. 9.50 | A 10 | Paral. poliom. | | + |
| [1] 9 | P. G. | 27 yrs | M | 17.11.52 | A 10 | Herpangina | + | + |
| 10 | Il. L. | 9 mths | M | 29. 7.53 | A 4 | Herpangina | | |
| 11 | T. R. | 4 yrs | F | 11. 4.53 | AR | Herpangina, tonsillitis | | |
| 15 | M. H. | 7 yrs | F | 21.10.52 | A 4 | Meningitis | | |
| 26 | ? | | | | B 3 | | | |
| 41 | T. L. | 32 yrs | M | 25. 6.52 | A 10 | Erythaema exsudat. multiforme | | |
| 42 | I. M. | 11 mths | F | 5. 8.52 | A 5 | Encephalitis | + | — |
| 45 | L. K. | 5 yrs | F | 30. 6.52 | BK | Meningitis | + | |
| 50 | In. L. | 7 yrs | F | 11. 8.53 | A 4 | Meningoencephalitis | + | + |
| 77 | H. H. | 1 yr | F | 28. 8.53 | AR | Herpangina | | |
| 80 | ? | | | | A 4 | Gastroenteritis | | |
| 89 | P. P. | 3 yrs | F | 12. 9.53 | AR | Encephalitis | | |
| 102 | ? | | | | B 1 | Gastroenteritis | | |
| 114 | Er. S. | 34 yrs | M | 25. 9.53 | A 10 | Minor illness | + | + |
| 115 | I. S. | 5 yrs | F | 26. 9.53 | A 10 | Herpangina | | |
| 116 | An. S. | 4 yrs | F | 23. 9.53 | A 10 | Herpangina | | |
| 117 | Ai. S. | 9 mths | F | 24. 9.53 | A 10 | Minor illness | | |
| 118 | Ev. S. | 31 yrs | F | 23. 9.53 | A 10 | Minor illness | | |
| [1] 129 | A. L. | 31 yrs | F | 28. 9.53 | A 10 | Herpangina | + | + |
| 141 | L. E. | 12 yrs | F | 29. 9.53 | E | Meningitis | + | |
| 158 | ? | | | | A 4 | Gastroenteritis | | |
| 167 | ? | | | | A 5 | | | |
| 169 | ? | | | | A 5 | | | |
| 175 | ? | | | | A 4 | | | |
| 202 | R. L. | 2 yrs | M | 23.11.53 | A 4 | Coeliacia | + | + |
| 228 | M. S. | 1 yrs | M | 7.12.53 | A 4 | Gastroenteritis | | |
| 237 | T. H. | 4 mths | F | 29.11.53 | A 4 | Gastroenteitis | + | + |
| 239 | M.K. | 1 yrs | M | 29.11.53 | A 4 | Gastroenteritis | + | + |
| 263 | V. K. | 26 yrs | F | 5.12.53 | A 4 | Healthy | | |
| 283 | J. A. | 16 yrs | M | 16.10.53 | A 4 | Minor illness | | |
| 289 | M. H. | 31 yrs | F | 22. 6.53 | A 10 | Healthy | | |
| [1] 318 | K. T. | 2 yrs | M | 22. 3.54 | A 10 | Minor illness | | |
| [1] 319 | E. T. | 28 yrs | F | 22. 3.54 | A 10 | Minor illness | + | + |

[1] Laboratory infections

## REFERENCES


1. Archetti, I., and Bortolo I, M.: Virus of the Coxsackie Group Isolated from a Dead Child. — (VI Congresso internazionale di microbiologia, Roma 1953).
2. Banker, D. D., and Melnick, J. L.: Isolation of Coxsackie Virus from North Alaskan Eskimos. — (Am. J. Hyg. 1951:*54:*383).
3. Beeman, E. A., Huebner, R. J., and Cole, R. M.: Studies of Coxsackie Viruses. Laboratory Aspects of Group A Viruses. — (Am. J. Hyg. 1952:*55:*83).
4. Beeman, E. A., and Huebner, R. J.: Evaluation of Serological Methods for Demonstrating Antibody Responses to Group A Coxsackie Viruses. — (J. Immunol. 1952:*68:*663).
5. Berger, E., Freudenberg, E., and Roulet, F.: Zur Kenntnis der Coxsackie virus Infektionen. — (Ann. Paediat. 1952:*179:*55).
6. Berger, E., and Roulet, F.: Beiträge zur Ausscheidung und Tierpathogenität des Coxsackie virus. — (Schweiz. Zschr. f. allg. Path. 1952:*15:*462).
7. Boak, R. A., Klaumann, B. F., and Ward, C. B.: Coxsackie Virus in Southern California; Isolation of a Strain from Stools of a Patient. — (California Med. 1952:*77:*187).
8. Breese, S. S. Jr., and Briefs, A.: Certain Physical Properties of a Herpangina Strain and a Pleurodynia Strain of Coxsackie Virus. — (Proc. Soc. Exper. Biol. & Med. 1953:*83:*119).
9. Briefs, A., Breese, S. S. Jr., Warren, J., and Huebner, F. J.: Physical Properties of Two Group A Coxsackie (Herpangina) Viruses when Propagated in Eggs and Mice as Determined by Ultracentrifugation and Electron Microscopy. — (J. Bact. 1952: *64:*237).
10. Brown, C. M., Liddle, D. C., and Tobin, J. O.: Bornholm Disease and Coxsackie Virus. — (Lancet, 1952:*I:*445).
11. Bury, H. S., and Tobin, J. O.: Further Outbreak of Bornholm Disease Associated with Coxsackie Virus. — (Lancet 1952:*II:*267)
12 Casals, J., and Olitsky, P. K.: Complement Fixation Tests with Some of the Viruses in the Coxsackie Group. — (Federation Proc. 1950:*9:*570).
13. Casals, J., Olitsky, P. K., and Murphy, L. C.: Hemagglutination and Complement Fixation with Type I and II Albany Strains of Coxsackie Virus. — (Proc. Soc. Exper. Biol. & Med. 1949:*72:*636).

14. Clark, E. M., Knowles, D. S., and Shimada, F. T.: Coxsackie Viruses in Urban Sewage. — (Canad. J. Pub. Health 1951:*42:*103).
15. Cole, R. M., Bell, J. A., Beeman, E A., and Huebner, R. J.: Studies of Coxsackie Viruses. Observations on Epidemiological Aspects of Group A Viruses. — (Am. J. Pub. Health 1951:*41:*1342).
16. O'Connor, J. R., and Morris, J. A.: Recovery of Texas-1 Type Coxsackie Virus from the Blood of Wild Rabbit and from Sewage Contaminating Rabbits Feeding Ground. — (Federation Proc. 1954:*13:*507).
17. Contreras, G., Barnett, V. H., and Melnick, J. L.: Indentification of Coxsackie Viruses by Immunological Methods and Their Classification Into 16 Antigenically Distinct Types. — (J. Immunol. 1952:*69:*395).
18. Contreras, G., and Melnick, J. L.: Growth Curves of Coxsackie Viruses in Mice, Including the Temporal Development of Complement Fixing Antigens. — (J. Immunol. 1953:*70:*473).
19. Contreras, G., and Melnick, J. L.: Complement Fixing Antibodies Against Coxsackie Viruses in Mice. — (J. Immunol. 1953:*70:*484).
20. Curnen, E. C.: Human Disease Associated with the Coxsackie Viruses. — (Bull. New York Acad. Med. 1950:*26:*335).
21. Curnen, E. C., and Melnick, J. L : Poliomyelitis and Coxsackie Viruses in Paralytic Poliomyelitis — (Pediatrics 1951:*8:*237).
22. Curnen, E. C., Shaw, E. W., and Melnick, J. L.: Disease Resembling Nonparalytic Poliomyelitis Associated with a Virus Pathogenic for Infant Mice. — (J.A.M.A. 1949:*141:*894).
23. Daae, A.: Epidemi i Drangedal af akut Muskelrheumatisme udbredt ved Smitte. — (Norsk mag. f. lægevidensk. 1872:*3:*409).
24. Dalldorf, G.: The Coxsackie Viruses. — (Bull. New York Acad. Med. 1950:*26:*329).
25. Dalldorf, G.: The Sparing Effect of Coxsackie Virus Infection on Experimental Poliomyelitis. — (J. Exper. Med. 1951:*94:*65).
26. Dalldorf, G.: Die Viren der Coxsackie-gruppe. (München med. Wchschr. 1952:*94:*2113).
27. Dalldorf, G., and Gifford, R.: Clinical and Epidemiological Observations of Coxsackie Virus Infection. — (New England J. Med. 1951: *244:*868).
28. Dalldorf, G., and Gifford, R.: Adaptation of Group B Coxsackie Virus to Adult Mouse Pancreas. — (J. Exper. Med. 1952:*96:*491).
29. Dalldorf, G., and Sickles, G. M.: An Unidentified Filtrable Agent Isolated from the Feces of Children with Paralysis. — (Science 1948:*108:*61).
30. Dalldorf, G., Sickles, G. M., Plager, H., and Gifford, R.: A Virus Recovered from Feces of »Poliomyelitis» Patients Pathogenic for Suckling Mice. — (J. Exper. Med. 1949:*89:*567).
31. Disney, M. E., Howard, E. M., Wood, B. S. B., and Findlay, G. M.: Bornholm Disease in Children. — (Brit. M. J. 1953:*II:*1351).

32. Findlay, G. M., and Howard, E. M.: Coxsackie Viruses and Bornholm Disease. — (Brit. M. J. 1950:*I:*1233).
33. Fox, T. G., and Murphy, A. M.: Herpangina with Isolation of the Virus. — (New Zealand M. J. 1953:*52:*257).
34. Fukumi, H., and Yokota, T.: Distribution of the Coxsackie Group of Viruses in Japan. — (Jap. J. Med. Sc. & Biol. 1953:*6:*201).
35. Gabinus, O., Gard, S., Johnsson, T., and Pöldre, A.: Studies on the Etiology of Epidemic Pleurodynia (Bornholm Disease). — Arch. f. die gesamte Virusforschung 1952:*V:*1).
36. Galpine, J. F., and Macrae, A. D.: An Outbreak of Benign Meningoencephalitis; Isolation of Coxsackie Virus. — (Lancet 1953:*1:*372).
37. Gard, S., and Johnsson, T.: Studies of Coxsackie Viruses. — (Acta path. et microbiol. Scandinav. 1952:*93:*332).
38. Geffen, T.: Bornholm Disease. — (Brit. M. J. 1951:*I:*1185).
39. Gifford, R., and Dalldorf, G.: The Morbid Anatomy of Experimental Coxsackie Virus Infection. — (Am. J. Path. 1951:*27:*1047).
40. Godenne, M. O., and Curnen, E. C.: Propagation of Coxsackie Virus on the Chorioallantoic Membrane of Embryonated Eggs. — (Proc. Soc. Exper. Biol. & Med. 1952:*80:*81).
41. Godman, G. C., Bunting, H., and Melnick, J. L.: The Histopathology of Coxsackie Virus Infection in Mice. I Morphologic Observations with Four Different Viral Types. — (Am. J. Path. 1951:*27:*1047).
42. Himmelweit, F., Findlay, G. M., and Howard, E. M.: The Size of Coxsackie Viruses as Estimated by Filtration through Gradocol Membranes. — (Brit. J. Exper. Path. 1950:*31:*809).
43. Homann, C.: Om en i Kragerö Lægedistrikt herskende smitsam Febersygdom. — (Norsk mag. f. lægevidensk. 1872:*3:*542).
44. Howes, D. W.: The Relationship of Age to the Susceptibility of Mice to Two Viruses of the Coxsackie Group. — (Australian J. Exper. Biol. & M. Sc. 1952:*30:*423).
45. Howitt, B. F.: Recovery of the Coxsackie Group of Viruses from human Sources. — (Proc. Soc. Exper. Biol. & Med. 1950:*73:*443).
46. Huebner, R. J., Armstrong, C., and Beeman, E. A.: Studies of Coxsackie Viruses. — (J.A.M.A. 1950:*144:*609).
47. Huebner, R. J., Coles, R. M., Beeman, E. A., Bell, J. A., and Peers, J. H.: Herpangina. Etiological Studies of a Specific Infection Disease. — (J.A.M.A. 1951:*145:*628).
48. Huebner, R. J., Ransom and Beeman, E. A.: Studies of Coxsackie Virus. Adaptation of a Strain to Chick Embryos. — (Pub. Health Rep. 1950:*65:*803).
49. Ivanovics, G., and Pintér, M.: Coxsackie Virus Infection in Hungary. — (Acta Med. Acad. Scient. Hungaricae 1953:*4:*157).
50. Johnsson, T.: Laboratory Infections with Coxsackie Viruses. — (Arch. f. die gesamte Virusforschung 1953:*V:*250).
51. Johnsson, T., and Lindahl, J.: Herpangina. — (Arch. f. die gesamte Virusforschung 1953:*V:*96).

52. Kenyon, H., Macrae, A. D., Dodds, R. J., and Galpine, J. F.: An Outbreak of Febrile Illness Associated with a Coxsackie Virus. — (Lancet 1952:*26:*153).

53. Kilbourne, E. D.: Diverse Manifestations of Infection with a Strain of Coxsackie Virus. — (Federation Proc. 1950:*9:*581).

54. Kilbourne, E. D., and Horsfall, F. L., Jr.: Lethal Infection with Coxsackie Virus of Adult Mice Given Cortisone. — (Proc. Soc. Exper. Biol. & Med. 1951:*77:*135).

55. Kraft, L. M., and Melnick, J. L.: Immunological Reactions of the Coxsackie Viruses. — (J. Exper. Med. 1950:*92:*483).

56. Kraft, L. M., and Melnick, J. L.: Complement Fixation Tests with Homologous and Heterologous Types of Coxsackie Virus in Man. — (J. Immunol. 1952:*68:*297).

57. Kraft, L. M., and Melnick, J. L.: Quantitative Studies of the Virus-host Relationship in Chimpanzees After Inapparent Infection with Coxsackie Viruses. II. The Development of Complement-fixing Antibodies. — (J. Exper. Med. 1953:*97:*401).

58. Kravies, L. P., Hummeler, K., Sigel, M. M., and Lecks, H. J.: Herpangina; Clinical and Laboratory Aspects of an Outbreak Caused by Group A Coxsackie Viruses. — (Pediatrics 1953:*11:*113).

59. Kunz, L. J., Richardson, S., and Pappenheimer, A. M.: Pancreatic Dicease in Mothers of Suckling Mice Infected with Connecticut No 5 Strain of Coxsackie Virus. — (Proc. Soc. Exper. Biol. & Med. 1952:*79:*488).

60. Lazarus, A. S., Johnston, E. A., and Galbraith, J. E.: An Outbreak of Epidemic Pleurodynia with Special Reference to the Laboratory diagnosis of Coxsackie Virus Infections. — (Am. J. Pub. Health 1952:*42:*20).

61. Lepine, P., Chaumont, L., and Blusson, J.: Sensibilité du mérion aux virus Coxsackie. — (Ann. Inst. Pasteur 1952:*82:*648).

62. Lepine, P., Desse, G., and Sautter, V.: Biopsies musculaires avec examen histologique et isolement du virus Coxsackie chez l'homme atteint de myalgie epidemique (Maladie de Bornholm). — (Bull. Acad. nat. med. 1952:*136:*66).

63. Levine, S., and Bernkopf, H.: The Occurrence of Coxsackie Viruses in Israel. — (Bull. of Research Council of Israel 1952:*II:*208).

64. von Magnus, H.: Isolering af tre virusstammer af Coxsackie gruppere fra patienter med meningeale symptomer. — (Ugesk. for Læger, 1949:*111:*1451).

65. Measroch, V., and Gear, J.: Studies in Poliomyelitis; the Isolation of a Coxsackie Like Virus from the Feces of Apparently Healthy Bantu Infants. — (South African M. J. 1951:*25:*421).

66. Melnick, J. L., and Curnen, E. C.: The Coxsackie Group. — (Viral and Rickettsial Infections of Man 1952:338. J. B. Lippincott, Phladelphia).

67. Melnick, J. L., Emmons, J., Coffey, J. H., and Schoof, H.:

Seasonal Distribution of Coxsackie Viruses in Urban Sewage and Flies. — (Am. J. Hyg. 1954:*59:*164).

68. Melnick, J. L., and Kaplan, A. S.: Dual Antibody Response to Coxsackie and Poliomyelitis Viruses in Patients with Paralytic Poliomyelitis. — (Proc. Soc. Exper. Biol. & Med. 1950:*74:*812).
69. Melnick, J. L., and Kaplan, A. S.: Quantitative Studies of the Virus-host Relationship in Chimpanzees after Inapparent Infection with Coxsackie Viruses. I. The Virus Carrier State and the Development of Neutralizing Antibodies. — (J. Exper. Med. 1953:*97:*367).
70. Melnick, J. L., Kaplan, A. S., Zabin, E., Contreras, G., and Larkum, N. W.: An Epidemic of Paralytic Poliomyelitis Characterized by Dual Infections with Poliomyelitis and Coxsackie Viruses. — (J. Exper. Med. 1951:*94:*471).
71. Melnick, J. L., and Ledinko, N.: Immunological Reactions of the Coxsackie Viruses. The Neutralisation Test: Technic and Application. — (J. Exper. Med. 1950:*92:*463).
72. Melnick, J. L., and Ledinko, N.: Social Serology: Antibody Levels in Normal Young Population during Epidemic of Poliomyelitis. — (Am. J. Hyg. 1952:*54:*354).
73. Melnick, J. L., Ledinko, N., Kaplan, A. S., and Kraft, L. M.: Ohio Strains of Virus Pathogenic for Infant Mice (Coxsackie group): Simultaneous Occurrence with Poliomyelitis Virus in Patients with »Summer Grippe». — (J. Exper. Med. 1950:*91:*185).
74. Melnick, J. L., and Penner, L. R.: The Survival of Poliomyelitis and Coxsackie Viruses following Their Ingestion by Flies. — (J. Exper. Med. 1952:*96:*255).
75. Melnick, J. L., Rhian, M., and Warren, J.: The Size of Coxsackie Viruses and Lansing Poliomyelitis Virus Determined by Sedimentation and Ultrafiltration. — (J. Immunol. 1951:*67:*151).
76. Melnick, J. L., Walton, M., and Myers, I. L.: Isolation of Coxsackie Virus during a Summer Outbreak of Acute Minor Illness. — (Pub. Health Rep. 1953:*68:*1178).
77. Melnick, J. L., and Ågren, K.: Poliomyelitis and Coxsackie Viruses Isolated from Normal Infants in Egypt. — (Proc. Soc. Exper. Biol. & Med. 1952:*81:*621).
78. Menut, G.: A propos de cinq cas d'affections neurologiques graves de l'enfant, dont un syndrome de Guillan-Barré; mise en evidence du virus Coxsackie. — (Arch. Franc. pédiat. 1952:*9:*978).
79. Morris, J. A.: Neutralizing Antibodies Against Viruses of Coxsackie Group in Sera of Wild Rabbits. — (Federation Proc. 1951:*10:*416).
80. Oker-Blom, N., and Nikkilä, E.: Purification of Coxsackie Virus by Use of Montmorillionite. — (Acta pathet. microbiol. Scandinav. Suppl. 1952:*93:*340).
81. Oker-Blom, N., and Nikkilä, E.: Purification of Certain Viruses by the Use of Bentonite. — (Ann. med. exper. et biol. Fenniae. To be published).

82. Oker-Blom, N. and Pohjanpelto, P.: The Occurrence of Coxsackie Group of Viruses in Finland. — (Ann. med. exper. et biol. Fenniae. 1953:*31:*166).

83. Pappenheimer, A. M., Kunz, L. J., and Richardson, S.: Passage of Coxsackie Virus (Conn. 5) in Adult Mice with Production of Pancreatic Disease. — (J. Exper. Med. 1951:*94:*45).

84. Parrott, R. H., Huebner, R. J., and Rice, E. C.: The Occurrence of Coxsackie Viruses in Hospitalized Poliomyelitis Patients. — (Clinical Proceedings of Children Hospital, Washington 1953:*9:*134).

85. Patz, I. M., Measroch, V., and Gear, J.: Bornholm Disease, Pleurodynia or Epidemic Myalgia; an Outbreak in the Transvaal Associated with Coxsackie Virus Infection. — (South African M. J. 1953:*27:*397).

86. Penttinen, K., and Halonen, P.: Studies on the Serological Diagnosis of Mumps. — (Ann. med. exper. et biol. Fenniae 1951:*20:*29).

87. Quigley, J. J.: Ultrafiltration and Ultracentrifugation Studies of Coxsackie Virus. — (Proc. Soc. Exper. Biol. & Med. 1949:*72:*434).

88. Rasmussen, A. F. Jr., Cline, F. A., and Borge, A. F.: Coxsackie Virus Infections in Northern Wisconsin. — (Wisconsin M. J. 1953:*52:*129).

89. Reed, L. J., and Muench, H.: A Simple Method of Estimating Fifty Per Cent Endpoints. — (Am. J. Hyg. 1938:*27:*493).

90. Rhodes, A. J., Clark, E. M., Knowles, D. S., Shimada, F. S., Ritchie, R. C., Donohue, W. L., Armstrong, M. P., Wilson, F. H. McLean, W. J., and Silverthorne, N.: Studies on Poliomyelitis in Ontario III. Further Observations on the Association of Coxsackie and Poliomyelitis Viruses. — (Canad. J. Pub. Health 1950: *41:*183).

91. Robinson, L. K.: Effect of Heat and pH on Strains of Coxsackie Virus. — (Proc. Soc. Exper. Biol. & Med. 1950:*75:*580).

92. Shaw, M.: Cultivation of Coxsackie Virus in Embryonated Eggs and in Chick Tissue Cultures. — (Proc. Soc. Exper. Biol. & Med. 1952: *79:*718).

93. Shaw, E. W., Melnick, J. L., and Curnen, E. C.: Infection of Laboratory Workers with Coxsackie Viruses. — (Ann. Int. Med. 1950:*33:* 32).

94. Silverthorne, N., Armstrong, M. P., Goodfellow, A. M., Roy, T. E., Ritchie, R. C., and Donohue, W. L.: Studies on Poliomyelitis in Ontario IV. Further Observations on the Spread of Poliomyelitis and Coxsackie Infections in Small Communities. — (Canad. M. Ass. J. 1951:*64:*309).

95. Slater, E. A., and Syverton, J. T.: The Cultivation of Coxsackie Virus. — (Proc. Soc. Exper. Biol. & Med. 1950:*74:*509).

96. Stanley, N. F., Dorman, D. C., and Ponsford, J.: Studies on Australian Strains of Coxsackie Virus. — (Australian J. Exper. Biol. & M. Sc. 1953:*31:*1).

97. Stulberg, C. S., Schapira, R., and Eudam, R.: Comparative Chaarc-

teristics of Two Coxsackie Viruses in Tissue Culture. — (J. Immunol. 1954:*72:*107).

98. Sulkin, S. E., and Wallis, H. C.: Effect of Ether on Certain Coxsackie Viruses. — (Proc. Soc. Exper. Biol. & Med. 1952:*81:*151).
99. Swain, R. H. A., and Mitchell, R. G.: The Isolation of a Coxsackie Virus from Two Cases of Bornholm Disease. — (Brit. M. J. 1953: *II:*1354).
100. Sylvest, E.: Epidemic myalgia: Bornholm Disease. — (London Oxford University Press 1934).
101. Taylor, R. M., Rizk, F., and Abdel-Kader: The Isolation of Coxsackie Like Viruses from Human Blood. — (J. Egypt. M. Ass. 1953:*36:*479).
102. Thordarson, O. T., Sigurdsson, B., and Grimsson, H.: Isolation of Coxsackie Virus from Patients with Epidemic Pleurodynia. — (J.A.M.A. 1953:*9:*814).
103. Travassos, J., Lobe, M. B., de Paola, D., and de Goés, P.: Isolation of the Viruses of the Coxsackie Group in Rio de Janeiro (Brazil). — (VI Congresso internazionale di microbiologia, Roma 1953).
104. Verlinde, J. D., and Tongeren, van H. A. E.: A Mixed Epidemic of Poliomyelitis and Bornholms Disease (Pleurodynia). — (Antonie van Leeuwenhoek 1952:*239:*18).
105. Vivell, O., and Gädeke, R.: Die Viren der Coxsackie-Gruppe. — (Erg. Hyg. Bakt. Immun. u. exper. Therapie 1952:*27:*512).
106. Warin, J. F., Davies, J. B. M., Sanders, F. K., and Vizoso, A. D.: Oxford Epidemic of Bornholm Disease 1951. — (Brit. M. J. 1953:*I:* 1345).
107. Webb, C. H., and Wolfe, S. G.: »Three Day Fever». An Acute Febrile Disease of Childhood. — (Am. J. Dis. Child. 1950:*80:*245).
108. Weller, T. H., Enders, J. F., Buckingham, M., and Finn, J. J.: The Etiology of Epidemic Pleurodynia: a Study of Two Viruses Isolated from Typical Outbreak. — (J. Immunol. 1950:*65:*337).
109. Weller, T. H., Robbins, F. C., Stoddard, M. B., and Florentino, G. L.: Propagation of Coxsackie Viruses in Cultures of Human Tissues. — (J. Immunol. 1953:*71:*92).
110. Windorfer, A., and Born, M.: Über die meningitische Form der Bornholmer Krankheit Meningitis myalgica. — (Deutsche med. Wchnschr. 1952:*77:*1012).
111. Wood, W., Clark, E. M., Shimada, F. T., Rhodes, A. J., Hutton, W. L., and Hutton, A. L.: Herpangina and Short-term Pyrexias of the Summer Months; Preliminary Observations on the Occurrence of These Syndromes in Southern Ontario. — (Canad. J. Pub. Health 1952:*43:*491).
112. Zahorsky, J.: Herpangina. — (South. M. J. 1920:*13:*87).

## ERRATA

From the numbers in the text which have reference to the bibliographical list at the end of the book (pp. 69—75) subtract one after 46 and two after 71.

*Page 35.* The controls in complementfixation test included titration of known immune serum.

*Page 41.* The Coxsackie virus was isolated from 3 per cent, not from 2 per cent, of the patients over 10 years of age.

*Page 42.* In the group of meningitis of unknown etiology one patient, not two, showed vesicles in the posterior part of the mouth.

*Page 52.* In table 11 the age of patient I.M. (No. 12) is 11 months, not 4 years.

*Page 63.* The Coxsackie virus could be isolated from 32 per cent, not from 31 per cent, of the cases of herpangina.

*Page 65.* The Coxsackie virus was isolated from 6 per cent, not from 5 per cent, of the subjects under 10 yeare of age and from 3 per cent, not from 2 per cent, of those over 10 years.

*Appendix* Patient In. L. (number 50) had herpangina in addition to meningoencephalitis.

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